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(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF ROCHESTER** [US/US]; 518 Hylan Building, Rochester, NY 14627 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **FEDEROFF, Howard** [US/US]; 375 Sandringham Drive, Rochester, NY 14618 (US). **BOWERS, William** [US/US]; 465 Trailwood Court, Webster, NY 14580 (US).

(74) Agents: **GOLDMAN, Michael, L.** et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 31051, Rochester, NY 14603-1051 (US).

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(54) Title: METHOD OF PRODUCING HERPES SIMPLEX VIRUS AMPLICONS, RESULTING AMPLICONS, AND THEIR USE

(57) Abstract: The present invention relates to a method for producing herpes simplex virus (HSV) amplicon particles which includes co-transfecting a host cell with the following: (i) an amplicon vector comprising an HSV origin of replication, an HSV cleavage/packaging signal, and a heterologous transgene expressible in a patient, (ii) one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals, and (iii) a vhs expression vector encoding a virion host shutoff protein; and then isolating HSV amplicon particles produced by the host cell, the HSV amplicon particles including the transgene. Also disclosed are a system and a kit for preparing HSV amplicon particles, HSV amplicon particles prepared according to the process of the present invention, and their use.

METHOD OF PRODUCING HERPES SIMPLEX VIRUS AMPLICONS, RESULTING AMPLICONS, AND THEIR USE

This application claims benefit of U.S. Provisional Application Serial No. 60/206,497, filed May 23, 2000, which is hereby incorporated by reference in its entirety.

The present invention was made, at least in part, with support from the National Institutes of Health Grant Nos. R01-NS36420 and R21-DK53160, and AFAR Research Grant. The U.S. government may have certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to an improved method for producing herpes simplex virus ("HSV") amplicons, the resulting HSV amplicons, and their use in gene therapy.

BACKGROUND OF THE INVENTION

The ability to deliver genes to the nervous system, and to manipulate their expression, may make possible the treatment of numerous neurological disorders. Unfortunately, gene transfer into the central nervous system ("CNS") presents several problems including the relative inaccessibility of the brain and the blood-brain-barrier, and that neurons of the postnatal brain are post-mitotic. The standard approach for somatic cell gene transfer, i.e., that of retroviral vectors, is not feasible for the brain, as retrovirally mediated gene transfer requires at least one cell division for integration and expression. A number of new vectors and non-viral methods have therefore been used for gene transfer in the CNS. Although the first studies of gene transfer in the CNS used an *ex vivo* approach, i.e., the transplantation of retrovirally-transduced cells, more recently several groups have also used an *in vivo* approach.

The *in vivo* approach was initially largely based on the use of the neurotropic herpes simplex virus ("HSV"), however, HSV vectors present several problems, including instability of expression and reversion to wild-type.

The genome of HSV-1 is about 150 kb of linear, double-stranded DNA, featuring about 70 genes. Many viral genes may be deleted without the virus losing its ability to propagate. The "immediately early" ("IE") genes are transcribed first. They encode trans-acting factors which regulate expression of other viral genes. The "early" ("E") gene products participate in replication of viral DNA. The late genes encode the structural components of the virion as well as proteins which turn on transcription of the IE and E genes or disrupt host cell protein translation.

After viral entry into the nucleus of a neuron, the viral DNA can enter a state of latency, existing as circular episomal elements in the nucleus. While in the latent state, its transcriptional activity is reduced. If the virus does not enter latency, or if it is reactivated, the virus produces numerous infectious particles, which leads rapidly to the death of the neuron. HSV-1 is efficiently transported between synaptically connected neurons, and hence can spread rapidly through the nervous system.

Two types of HSV vectors previously have been utilized for gene transfer into the nervous system. Recombinant HSV vectors involve the removal of an immediate-early gene within the HSV genome (ICP4, for example), and replacement with the gene of interest. Although removal of this gene prevents replication and spread of the virus within cells which do not complement for the missing HSV protein, all of the other genes within the HSV genome are retained. Replication and spread of such viruses *in vivo* is thereby limited, but expression of viral genes within infected cells continues. Several of the viral expression products may be directly toxic to the recipient cell, and expression of viral genes within cells expressing MHC antigens can induce harmful immune reactions. In addition, nearly all adults harbor latent herpes simplex viruses within neurons, and the presence of recombinant HSV vectors could result in recombinations which can produce an actively replicating wild-type virus. Alternatively, expression of viral genes from the recombinant vector within a cell harboring a latent virus might promote reactivation of the virus. Finally, long-term expression from the recombinant HSV vector in the CNS has not been reliably demonstrated. It is likely that, except for conditions in

which latency is induced, the inability of HSV genomes to integrate within host DNA results in susceptibility to degradation of the vector DNA.

In an attempt to circumvent the difficulties inherent in the recombinant HSV vector, defective HSV vectors were employed as gene transfer vehicles within the nervous system. The defective HSV vector is a plasmid-based system, whereby a plasmid vector (termed an amplicon) is generated which contains the gene of interest and two cis-acting HSV recognition signals. These are the origin of DNA replication and the cleavage packaging signal. These sequences encode no HSV gene products. In the presence of HSV proteins provided by a helper virus, the amplicon is replicated and packaged into an HSV coat. This vector therefore expresses no viral gene products within the recipient cell, and recombination with or reactivation of latent viruses by the vector is limited due to the minimal amount of HSV DNA sequence present within the defective HSV vector genome. The major limitation of this system, however, is the inability to eliminate residual helper virus from the defective vector stock. The helper virus is often a mutant HSV which, like the recombinant vectors, can only replicate under permissive conditions in tissue culture. The continued presence of mutant helper HSV within the defective vector stock, however, presents problems which are similar to those enumerated above in regard to the recombinant HSV vector. This would therefore serve to limit the usefulness of the defective HSV vector for human applications.

While HSV vectors of reduced toxicity and replication ability have been suggested, they can still mutate to a more dangerous form, or activate a latent virus, and, since the HSV does not integrate, achieving long-term expression would be difficult.

To avoid the difficulties raised with the use of helper viruses, newer methods of packaging have been developed that result in "helper virus-free" amplicon stocks (Fraefel et al., "Helper virus-free transfer of herpes simplex virus type 1 plasmid vectors into neural cells," J. Virol., **70**:7190-7197 (1996); Stavropoulos and Strathdee, "An enhanced packaging system for helper-dependent herpes simplex virus vectors," J. Virol., **72**:7137-43 (1998)). Stocks produced by these means, however, are typically of low titer (approximately 10^5 expression units/ml or less), allowing for only modest *in vitro* experimentation. Such low titers discourage investigators from

performing the large animal studies required to develop and assess amplicon-directed therapies in mammals, including humans.

The present invention is directed to overcoming these deficiencies in the art.

SUMMARY OF THE INVENTION

A first aspect of the present invention relates to a method for producing herpes simplex virus ("HSV") amplicon particles, which includes co-transfecting a host cell with the following: (i) an amplicon vector comprising an HSV origin of replication, an HSV cleavage/packaging signal, and a heterologous transgene expressible in a patient, (ii) one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals, and (iii) a vhs expression vector encoding a virion host shutoff protein; and then isolating HSV amplicon particles produced by the host cell, the HSV amplicon particles including the transgene.

A second aspect of the present invention relates to HSV amplicon particles produced according to the method of the present invention.

A third aspect of the present invention relates to a system for preparing HSV amplicon particles which includes: an amplicon vector comprising an HSV origin of replication, an HSV cleavage/packaging signal, and a transgene insertion site; one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals; and a vhs expression vector encoding a virion host shutoff protein; wherein upon introduction of the system into a host cell, the host cell produces herpes simplex virus amplicon particles.

A fourth aspect of the present invention relates to a kit for preparing HSV amplicon particles which includes: an amplicon vector comprising an HSV origin of replication, an HSV cleavage/packaging signal, and a transgene insertion site; one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals; a vhs expression vector encoding an virion host shutoff protein; a population of host cells susceptible to transfection by the amplicon vector, the vhs expression vector, and the one or more vectors; and

directions for transfecting the host cells under conditions to produce HSV amplicon particles.

A fifth aspect of the present invention relates to a method of treating a neurological disease or disorder which includes providing HSV amplicon particles of the present invention that include a transgene encoding a therapeutic transgene product and exposing neural or pre-neural cells of a patient to the HSV amplicon particles under conditions effective for infective transformation of the neural or pre-neural cells, wherein the therapeutic transgene product is expressed *in vivo* in the neural or pre-neural cells, thereby treating the neurological disease or disorder.

A sixth aspect of the present invention relates to a method of inhibiting development of a neurological disease or disorder which includes providing HSV amplicon particles of the present invention that include a transgene encoding a therapeutic transgene product and exposing neural or pre-neural cells of a patient susceptible to development of a neurological disease or disorder to the HSV amplicon particles under conditions effective for infective transformation of the neural or pre-neural cells of the patient, wherein the therapeutic transgene product is expressed *in vivo* in the neural or pre-neural cells, thereby inhibiting development of the neurological disease or disorder.

A seventh aspect of the present invention relates to a method of expressing a therapeutic gene product in a patient which includes providing HSV amplicon particles of the present invention that include a transgene encoding a therapeutic transgene product and exposing patient cells to the HSV amplicon particles under conditions effective for infective transformation of the cells, wherein the therapeutic transgene product is expressed *in vivo* in transformed cells.

In an effort to enhance amplicon titers, the present invention involves introduction *in trans* of a vector including a sequence which encodes a virion host shutoff protein. Co-transfection of this plasmid, specifically one containing the HSV virion host shutoff ("vhs") protein-encoding gene *UL41*, with the amplicon and packaging reagents results in a 10-fold higher amplicon titer and stocks that do not exhibit the pseudotransduction phenomenon. To further enhance packaging efficiency, the HSV transcriptional activator VP16 was introduced into packaging cells prior to the packaging components. Pre-loading of packaging cells with VP16 led to an additional enhancement of amplicon titers, an effect that did not occur in the

absence of vhs. Increased helper virus-free amplicon titers resulting from these modifications will make *in vivo* transduction experiments more feasible.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-B are maps of suitable amplicon vectors. Figure 1A is a map of the empty amplicon vector pHSVlac, which includes the HSV-1 *a* segment (cleavage/packaging or *pac* signal), the HSV-1 *c* region (origin of replication), an ampicillin resistance marker, and an *E. coli lacZ* marker under control of HSV *IE4* promoter and SV40 polyadenylation signal. Figure 1B illustrates insertion of a transgene into *Bam*HI site adjacent the HSV-1 *a* segment, forming pHSVlac/trans.

Figures 2A-B are maps of the HSV-1 genome and the overlapping 5 cosmid set C6Δ*a*48Δ*a* (cos6Δ*a*, cos28, cos14, cos56, and cos48Δ*a*) (Fraefel et al., "Helper virus-free transfer of herpes simplex virus type 1 plasmid vectors into neural cells," J. Virol., **70**:7190-7197 (1996), which is hereby incorporated by reference in its entirety). In the HSV-1 genome of Figure 2A, only the *IE4* gene, ori_S, and ori_L are shown. The *a* sequences, which contain the cleavage/packaging sites, are located at the junction between long and short segments and at both termini. In Figure 2B, the deleted *a* sequences in cos6Δ*a* and cos48Δ*a* are indicated by "X".

Figure 3 is a map of the HSV bacterial artificial chromosome (HSV-BAC).

Figures 4A is a map of pBSKS(vhs), a plasmid vector which includes the HSV-1 vhs coding region (SEQ ID No: 3) operatively coupled to its native transcriptional control elements. Figures 4B-C show the nucleotide sequence of a 4.3 kb fragment of the HSV-1 genome which contains the vhs gene with its native promoter and polyadenylation signal sequences (SEQ ID No: 1). The vhs coding sequence is underlined.

Figure 5 is a map of $pGRE_5vp16$, a plasmid vector which includes five glucocorticoid responsive elements located upstream of a adenovirus major late promoter having a TATA box, an HSV vp16 coding sequence (SEQ ID No: 5), and an SV40 polyadenylation signal. The plasmid also includes an ampicillin resistance marker.

Figures 6A-B are graphs which illustrate the effect of vhs expression on helper virus-free amplicon packaging titers. The β-galactosidase-expressing (LacZ) HSV amplicon vector (HSVlac) was packaged in the absence or presence of pBS(vhs) by either the cosmid-(Figure 6A) or BAC-based (Figure 6B) helper virus-free production strategy. This pBS(vhs) plasmid possesses the *vhs* open reading frame as well as its entire 5' and 3' regulatory sequences. Amplicon stocks were harvested and used to transduce NIH 3T3 cells, and titers were determined one day later via enumeration of LacZ-positive cells. Titer data are expressed as blue-forming units per milliliter (bfu/ml) and error bars represent standard deviation.

Figures 7A-G are images which illustrate the *in vitro* and *in vivo* analysis of vhs-mediated enhancement of helper-free amplicon titers. Ten microliters of BAC-packaged HSVlac produced without (Figure 7A) or in the presence of pBS(vhs) (Figure 7B) was used to transduce NIH 3T3 fibroblasts. LacZ-positive cells were visualized by X-gal histochemistry and images were digitally acquired. Ten microliters of BAC-packaged HSVPrPUC/CMVegfp produced either without (Figure 7C) or in the presence of pBS(vhs) (Figure 7D) was used to transduce NIH 3T3 fibroblasts. Green fluoresecent protein (GFP)-positive cells were visualized with a fluorescent microscope and images digitally acquired. Three microliters of the same virus samples packaged either in the absence (Figure 7E) or in the presence of pBS(vhs) (Figure 7F) was stereotactically delivered into the striata of C57BL/6 mice. Animals were sacrificed four days later and prepared for visualization and quantitation of GFP-positive cells. Images used for morphological analyses were digitally acquired at 200x magnification on 40-μm sections. All compartments were processed for cell counting and GFP-positive cell numbers reflect cell counts throughout the entire injection site (Figure 7G). The asterisk indicates a statistically significant difference ($p < 0.001$) between amplicon stocks packages with BAC alone and those packaged with BAC in the presence of pBS(vhs).

Figures 8A-D are graphs illustrating the effects of vhs presence during amplicon packaging on freeze/fracture stability and thermostability. BAC-packaged HSVPrPUC/CMVegfp stocks produced in the presence (circles) or absence (squares) of vhs were incubated at 0°C (Figure 8A), 22°C (Figure 8B), or 37°C (Figure 8C) for varying time periods. At 0, 30, 60, 120, and 180 minutes following initiation of the incubations, aliquots were removed, titered on NIH 3T3 cells, and expression titer

data represented as green-forming units per milliliter. Another set of HSVPrPUC/CMVegfp stocks were subjected to a series of freeze-thaw cycles to determine sensitivity of viral particles to freeze fracture. Following each cycle, aliquots were removed, titered on NIH 3T3 cells, and expression titer data represented as green-forming units per milliliter (gfu/ml; Figure 8D).

Figures 9A-C illustrate the effect of the pre-loading of packaging cells with VP16 on enhancement of amplicon expression titers only in presence of vhs. BHK cells were plated and 6 hours later, were transfected with a glucocorticoid-regulated VP16 expression vector ($pGRE_5vp16$). A subset of cultures received 100 nM dexamethasone following the VP16 plasmid transfection. The following day, HSVlac, a β-galactosidase-expressing amplicon, was cosmid- (Figure 9A) or BAC-packaged (Figure 9B) in the absence or presence of the pBS(vhs) plasmid using the modified BHK cultures. Resultant amplicon stocks were titered on NIH 3T3 cells using X-gal histochemistry and titers represented as blue-forming units per milliliter (bfu/ml; Figures 9A-B). Error bars represent standard deviation. Western blot analysis was performed to determine levels of VP16 expression in various combinations of helper virus-free packaging components (Figure 9C). Lysates were harvested 48 h following introduction of BAC reagent. Lane designations are the following: BHK cells alone (Lane 1); BHK cells transfected with BAC only (Lane 2); BHKs transfected with $pGRE_5vp16$ 24 h prior to BAC transfection in the absence of dexamethasone (Lane 3); and BHKs transfected with $pGRE_5vp16$ 24 h prior to BAC transfection in the presence of 100 nM dexamethasone (Lane 4). The 65-kDa VP16 protein was detected using a VP16-specific monoclonal antibody and goat anti-mouse secondary antibody in combination with a chemiluminescent detection kit.

Figure 10 is a graph illustrating that the virion-incorporated amplicon genome levels are enhanced by ectopic expression of VP16. BAC-packaged HSVlac stocks prepared in the presence or absence of VP16 and/or vhs were analyzed for levels of genome content using a "real-time" quantitative PCR technique. Nanogram quantities of vector genome were assayed for each sample and data were expressed as detected amplicon genome per milliliter. Error bars represent standard deviation.

Figure 11 is a graph illustrating the virion-incorporated amplicon genome levels are enhanced by ectopic expression of VP16. BAC-packaged HSVlac stocks prepared in the presence or absence of VP16 and/or vhs were analyzed for

amplicon titer (bfu/ml) using a "real-time" analysis. Error bars represent standard deviation.

Figure 12 is a graph illustrating that amplicon stock-mediated cytotoxicity is not increased by additional expression of vhs and VP16 during packaging. BAC-packaged HSVlac stocks prepared in the presence or absence of VP16 and/or vhs were analyzed on confluent monolayers of NIH 3T3 cells for elicited cytotoxicity as determined by an LDH release-based assay. Two of the packaging samples that received $pGRE_5vp16$ were also treated with 100 nM dexamethasone 24 hours prior to the packaging transfection. Equivalent expression units of virus from each packaging sample were used in the transductions. Viability data were represented as normalized cell viability index.

Figure 13 is a scanning electron micrograph image of purified helper-virus free HSV-1 amplicon virion stocks prepared using a negative staining technique. Arrows denote individual amplicon particles.

Figure 14 is an image of a two-dimension gel for polypeptide analysis of virion particle stock prepared using helper virus-free procedure according to the present invention. Individual spots have been numbered. See Table 2, Example 4, for spot numbering and measurements.

Figure 15 is an image of a two-dimension gel for polypeptide analysis of virion particle stock prepared using helper virus procedure which is known in the art. Individual spots have been numbered. See Table 2, Example 4, for spot numbering and measurements.

Figures 16A-B are difference images of gels shown in Figures 14 and 15, showing spots which are increased in Figure 15 as compared to Figure 14. Figure 16B is an enlarged view of the most crowded region. See Table 2, Example 4, for spot numbering and measurements.

Figures 17A-C are difference images of gels shown in Figures 14 and 15, showing spots which are decreased in Figure 15 as compared to Figure 14. Figures 17B-C are enlarged views of the two most crowded regions. See Table 2, Example 4, for spot numbering and measurements.

DETAILED DESCRIPTION OF THE INVENTION

One aspect of the present invention relates to a method for producing herpes simplex virus (HSV) amplicon particles. This method is carried out by co-transfecting a host cell with several vectors and then isolating HSV amplicon particles produced by the host cell. The vectors used to transfect the host cell include: (i) an amplicon vector comprising an HSV origin of replication, an HSV cleavage/packaging signal, and a heterologous transgene expressible in a patient; (ii) one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals; and (iii) a vhs expression vector encoding a virion host shutoff protein. As a result of the transgene being included in the HSV amplicon vector, the HSV amplicon particles include the transgene.

The amplicon vector is any HSV amplicon vector which includes an HSV origin of replication, an HSV cleavage/packaging signal, and a heterologous transgene expressible in a patient. The amplicon vector can also include a selectable marker gene and an antibiotic resistance gene.

The HSV cleavage/packaging signal can be any suitable cleavage/packaging signal such that the vector can be packaged into a particle that is capable of adsorbing to a cell (i.e., which is to be transformed). A suitable packaging signal is the HSV-1 *a* segment located at approximately nucleotides 127-1132 of the *a* sequence of the HSV-1 virus or its equivalent (Davison et al., "Nucleotide sequences of the joint between the L and S segments of herpes simplex virus types 1 and 2," J. Gen. Virol. **55**:315-331 (1981), which is hereby incorporated by reference in its entirety).

The HSV origin of replication can be any suitable origin of replication which allows for replication of the amplicon vector in the host cell which is to be used for replication and packaging of the vector into the HSV amplicon particles. A suitable origin of replication is the HSV-1 *c* region which contains the HSV-1 ori_s segment located at approximately nucleotides 47-1066 of the HSV-1 virus or its equivalent (McGeogh et al., Nucl. Acids Res. **14**:1727-1745 (1986), which is hereby incorporated by reference in its entirety). Origin of replication signals from other related viruses (e.g., HSV-2) can also be used.

Selectable marker genes are known in the art and include, without limitation, galactokinase, beta-galactosidase, chloramphenicol acetyltransferase, beta-lactamase, green fluorescent protein ("gfp"), alkaline phosphate, etc.

Antibiotic resistance genes are known in the art and include, without limitation, ampicillin, streptomycin, spectromycin, etc.

A number of suitable empty amplicon vectors have previously been described in the art, including without limitation: pHSVlac (ATCC Accession 40544; U.S. Patent No. 5,501,979 to Geller et al.; Stavropoulos and Strathdee, "An enhanced packaging system for helper-dependent herpes simplex virus vectors," J. Virol., **72**:7137-43 (1998), which are hereby incorporated by reference in their entirety) and pHENK (U.S. Patent No. 6,040,172 to Kaplitt et al., which is hereby incorporated by reference. The pHSVlac vector includes the HSV-1 *a* segment, the HSV-1 *c* region, an ampicillin resistance marker, and an *E. coli lacZ* marker. The pHENK vector include the HSV-1 *a* segment, an HSV-1 *ori* segment, an ampicillin resistance marker, and an *E. coli lacZ* marker under control of the promoter region isolated from the rat preproenkephalin gene (i.e., a promoter operable in brain cells).

These empty amplicon vectors can be modified by introducing therein, at an appropriate restriction site, either a complete transgene which has already been assembled or a coding sequence can be ligated into an empty amplicon vector which already contains appropriate regulatory sequences (promoter, enhancer, polyadenylation signal, transcription terminator, etc.) positioned on either side of the restriction site where the coding sequence is to be inserted, thereby forming the transgene upon ligation. Alternatively, when using the pHSVlac vector, the *lacZ* coding sequence can be excised using appropriate restriction enzymes and replaced with a coding sequence for the transgene.

The use of restriction enzymes for cutting DNA and the use of DNA ligase to ligate together two or more DNA molecules can be performed using conventional molecular genetic manipulation for subcloning gene fragments, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989); Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding editions); and U.S. Patent No. 4,237,224 issued to Cohen and Boyer, which are hereby incorporated by reference in their entirety.

Suitable transgenes will include one or more appropriate promoter elements which are capable of directing the initiation of transcription by RNA polymerase, optionally one or more enhancer elements, and suitable transcription terminators or polyadenylation signals.

Basically, the promoter elements should be selected such that the promoter will be operable in the cells of the patient which are ultimately intended to be transformed (i.e., during gene therapy). A number of promoters have been identified which are capable of regulating expression within a broad range of cell types. These include, without limitation, HSV immediate-early 4/5 (IE4/5) promoter, cytomegalovirus ("CMV") promoter, SV40 promoter, and β-actin promoter. Likewise, a number of other promoters have been identified which are capable of regulating expression within a narrow range of cell types. These include, without limitation, neural-specific enolase (NSE) promoter, tyrosine hydroxylase (TH) promoter, GFAP promoter, preproenkephalin (PPE) promoter, myosin heavy chain (MHC) promoter, insulin promoter, cholineacetyltransferase (ChAT) promoter, dopamine ß-hydroxylase (DBH) promoter, calmodulin dependent kinase (CamK) promoter, c-fos promoter, c-jun promoter, vascular endothelial growth factor (VEGF) promoter, erythropoietin (EPO) promoter, and EGR-1 promoter.

The transcription termination signal should, likewise, be selected such that they will be operable in the cells of the patient which are ultimately intended to be transformed. Suitable transcription termination signals include, without limitation, polyA signals of HSV genes such as the vhs polyadenylation signal, SV40 polyA signal, and CMV IE1 polyA signal.

When used for gene therapy, the transgene encodes a therapeutic transgene product, which can be either a protein or an RNA molecule.

Therapeutic RNA molecules include, without limitation, antisense RNA, inhibitory RNA (RNAi), and an RNA ribozyme. The RNA ribozyme can be either *cis* or *trans* acting, either modifying the RNA transcript of the transgene to afford a functional RNA molecule or modifying another nucleic acid molecule. Exemplary RNA molecules include, without limitation, antisense RNA, ribozymes, or RNAi to nucleic acids for huntingtin, alpha synuclein, scatter factor, amyloid precursor protein, p53, VEGF, etc.

Therapeutic proteins include, without limitation, receptors, signaling molecules, transcription factors, growth factors, apoptosis inhibitors, apoptosis promoters, DNA replication factors, enzymes, structural proteins, neural proteins, and histone or non-histone proteins. Exemplary protein receptors include, without limitation, all steroid/thyroid family members, nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophins 3 and 4/5, glial derived neurotrophic factor (GDNF), cilary neurotrophic factor (CNTF), persephin, artemin, neurturin, bone morphogenetic factors (BMPs), c-ret, gp130, dopamine receptors (D1-D5), muscarinic and nicotinic cholinergic receptors, epidermal growth factor (EGF), insulin and insulin-like growth factors, leptin, resistin, and orexin. Exemplary protein signaling molecules include, without limitation, all of the above-listed receptors plus MAPKs, ras, rac, ERKs, NFKß, GSK3ß, AKT, and PI3K. Exemplary protein transcription factors include, without limitation, p300, CBP, HIF-1alpha, NPAS1 and 2, HIF-1ß, p53, p73, nurr 1, nurr 77, MASHs, REST, and NCORs. Exemplary neural proteins include, without limitation, neurofilaments, GAP-43, SCG-10, etc. Exemplary enzymes include, without limitation, TH, DBH, aromatic aminoacid decarboxylase, parkin, unbiquitin E3 ligases, ubiquitin conjugating enzymes, cholineacetyltransferase, neuropeptide processing enzymes, dopamine, VMAT and other catecholamine transporters. Exemplary histones include, without limitation, H1-5. Exemplary non-histones include, without limitation, ND10 proteins, PML, and HMG proteins. Exemplary pro- and anti-apoptotic proteins include, without limitation, bax, bid, bak, bcl-xs, bcl-xl, bcl-2, caspases, SMACs, and IAPs.

The one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals can either be in the form of a set of vectors or a single bacterial-artificial chromosome ("BAC"), which is formed, for example, by combining the set of vectors to create a single, double-stranded vector. Preparation and use of a five cosmid set is disclosed in (Fraefel et al., "Helper virus-free transfer of herpes simplex virus type 1 plasmid vectors into neural cells," J. Virol., **70**:7190-7197 (1996), which is hereby incorporated by reference in its entirety). Ligation of the cosmids together to form a single BAC is disclosed in Stavropoulos and Strathdee, "An enhanced packaging system for helper-dependent herpes simplex virus vectors," J. Virol., **72**:7137-43 (1998), which is hereby incorporated by reference in its entirety). The BAC described in Stavropoulos

and Strathdee includes a *pac* cassette inserted at a *Bam*HI site located within the *UL41*coding sequence, thereby disrupting expression of the HSV-1 virion host shutoff protein.

By "essential HSV genes", it is intended that the one or more vectors include all genes which encode polypeptides that are necessary for replication of the amplicon vector and structural assembly of the amplicon particles. Thus, in the absence of such genes, the amplicon vector is not properly replicated and packaged within a capsid to form an amplicon particle capable of adsorption. Such "essential HSV genes" have previously been reported in review articles by Roizman ("The Function of Herpes Simplex Virus Genes: A Primer for Genetic Engineering of Novel Vectors," Proc. Natl. Acad. Sci. USA **93**:11307-11312 (1996);"HSV Gene Functions: What Have We Learned That Could Be Generally Application to its Near and Distant Cousins?" Acta Virologica **43**(2-3):75-80 (1999), which are hereby incorporated by reference in their entirety. Another source for identifying such essential genes is available at the Internet site operated by the Los Alamos National Laboratory, Bioscience Division, which reports the entire HSV-1 genome and includes a table identifying the essential HSV-1 genes. The genes currently identified as essential are listed in Table 1 below.

Table 1: Essential HSV-1 Genes

Gene*	Protein(Function)	Genbank I.D. No.	Genbank Accession No.**
UL1	virion glycoprotein L (gL)	136775	CAA32337
UL5	component of DNA helicase-primase complex	74000	CAA32341
UL6	minor capsid protein	136794	CAA32342
UL7	unknown	136798	CAA32343
UL8	DNA helicase/primase complex associated protein	136802	CAA32344
UL8.5	unknown***	-	-
UL9	ori-binding protein	136806	CAA32345
UL15	DNA cleavage/packaging protein	139646	CAA32330
UL17	tegument protein	136835	CAA32329
UL18	capsid protein, VP23	139191	CAA32331
UL19	major capsid protein, VP5	137571	CAA32332
UL22	virion glycoprotein H, gH	138315	CAA32335
UL25	DNA packaging virion protein	136863	CAA32317
UL26	serine protease, self-cleaves to form VP21 & VP24	139233	CAA32318
UL26.5	capsid scaffolding protein, VP22a	1944539	CAA32319
UL27	virion glycoprotein B, gB	138194	CAA32320
UL28	DNA cleavage and packaging protein, ICP18.5	124088	CAA32321
UL29	single-stranded DNA binding protein, ICP8	118746	CAA32322
UL30	DNA polymerase	118878	CAA32323
UL31	UL34-associated nuclear protein	136875	CAA32324
UL32	cleavage and packaging protein	136879	CAA32307
UL33	capsid packaging protein	136883	CAA32308
UL34	membrane-associated virion protein	136888	CAA32309
UL36	very large tegument protein, ICP1/2	135576	CAA32311
UL37	tegument protein, ICP32	136894	CAA32312
UL38	capsid protein, VP19C	418280	CAA32313
UL42	DNA polymerase accessory protein	136905	CAA32305
UL48	alpha trans-inducing factor, VP16	114359	CAA32298
UL49	putative microtubule-associated protein, VP22	136927	CAA32299
UL49.5	membrane-associated virion protein	1944541	CAA32300
UL52	component of DNA helicase/primase complex	136939	CAA32288
UL54	regulation and transportation of RNA, ICP27	124180	CAA32290
α4 (RS1)	positive and negative gene regulator, ICP4	124141	CAA32286 CAA32278
US6	virion glycoprotein D, gD	73741	CAA32283

* The complete genome of HSV-1 is reported at Genbank Accession No. X14112, which is hereby incorporated by reference in its entirety.

** Each of the listed Accession Nos. which report an amino acid sequence for the encoded proteins is hereby incorporated by reference in its entirety.

*** UL8.5 maps to a transcript which overlaps and is in frame with the carboxyl terminal of UL9 (Baradaran et al., "Transcriptional analysis of the region of the herpes simplex virus type 1 genome containing the UL8, UL9, and UL10 genes and identification of a novel delayed-early gene product, OBPC," J. Virol. **68**(7):4251-4261 (1994), which is hereby incorporated by reference in its entirety).

The vhs vector can encode a virion host shutoff ("vhs") protein which is effective in regulating host cell transcription and translation activities. The vhs vector includes a DNA molecule encoding a vhs protein, which DNA molecule is operably coupled 5' to a promoter which is functional in the host cell and 3' to a transcription terminator which also is functional in the host cell.

One suitable vhs protein is the human herpesvirus 1 vhs protein, which has an amino acid sequence according to SEQ ID No: 2 as follows:

```
Met Gly Leu Phe Gly Met Met Lys Phe Ala His Thr His His Leu Val
 1               5                  10                  15

Lys Arg Arg Gly Leu Gly Ala Pro Ala Gly Tyr Phe Thr Pro Ile Ala
            20                  25                  30

Val Asp Leu Trp Asn Val Met Tyr Thr Leu Val Val Lys Tyr Gln Arg
        35                  40                  45

Arg Tyr Pro Ser Tyr Asp Arg Glu Ala Ile Thr Leu His Cys Leu Cys
    50                  55                  60

Arg Leu Leu Lys Val Phe Thr Gln Lys Ser Leu Phe Pro Ile Phe Val
65                  70                  75                  80

Thr Asp Arg Gly Val Asn Cys Met Glu Pro Val Val Phe Gly Ala Lys
                85                  90                  95

Ala Ile Leu Ala Arg Thr Thr Ala Gln Cys Arg Thr Asp Glu Glu Ala
            100                 105                 110

Ser Asp Val Asp Ala Ser Pro Pro Pro Ser Pro Ile Thr Asp Ser Arg
        115                 120                 125

Pro Ser Ser Ala Phe Ser Asn Met Arg Arg Arg Gly Thr Ser Leu Ala
    130                 135                 140

Ser Gly Thr Arg Gly Thr Ala Gly Ser Gly Ala Ala Leu Pro Ser Ala
145                 150                 155                 160

Ala Pro Ser Lys Pro Ala Leu Arg Leu Ala His Leu Phe Cys Ile Arg
                165                 170                 175

Val Leu Arg Ala Leu Gly Tyr Ala Tyr Ile Asn Ser Gly Gln Leu Glu
            180                 185                 190

Ala Asp Asp Ala Cys Ala Asn Leu Tyr His Thr Asn Thr Val Ala Tyr
        195                 200                 205

Val Tyr Thr Thr Asp Thr Asp Leu Leu Leu Met Gly Cys Asp Ile Val
    210                 215                 220

Leu Asp Ile Ser Ala Cys Tyr Ile Pro Thr Ile Asn Cys Arg Asp Ile
225                 230                 235                 240
```

```
Leu Lys Tyr Phe Lys Met Ser Tyr Pro Gln Phe Leu Ala Leu Phe Val
                245                 250                 255

Arg Cys His Thr Asp Leu His Pro Asn Asn Thr Tyr Ala Ser Val Glu
            260                 265                 270

Asp Val Leu Arg Glu Cys His Trp Thr Pro Pro Ser Arg Ser Gln Thr
        275                 280                 285

Arg Arg Ala Ile Arg Arg Glu His Thr Ser Ser Arg Ser Thr Glu Thr
    290                 295                 300

Arg Pro Pro Leu Pro Pro Ala Ala Gly Gly Thr Glu Thr Arg Val Ser
305                 310                 315                 320

Trp Thr Glu Ile Leu Thr Gln Gln Ile Ala Gly Gly Tyr Glu Asp Asp
                325                 330                 335

Glu Asp Leu Pro Leu Asp Pro Arg Asp Val Thr Gly Gly His Pro Gly
            340                 345                 350

Pro Arg Ser Ser Ser Ser Glu Ile Leu Thr Pro Pro Glu Leu Val Gln
        355                 360                 365

Val Pro Asn Ala Gln Leu Leu Glu Glu His Arg Ser Tyr Val Ala Asn
    370                 375                 380

Pro Arg Arg His Val Ile His Asp Ala Pro Glu Ser Leu Asp Trp Leu
385                 390                 395                 400

Pro Asp Pro Met Thr Ile Thr Glu Leu Val Glu His Arg Tyr Ile Lys
                405                 410                 415

Tyr Val Ile Ser Leu Ile Gly Pro Lys Glu Arg Gly Pro Trp Thr Leu
            420                 425                 430

Leu Lys Arg Leu Pro Ile Tyr Gln Asp Ile Arg Asp Glu Asn Leu Ala
        435                 440                 445

Arg Ser Ile Val Thr Arg His Ile Thr Ala Pro Asp Ile Ala Asp Arg
    450                 455                 460

Phe Leu Glu Gln Leu Arg Thr Gln Ala Pro Pro Pro Ala Phe Tyr Lys
465                 470                 475                 480

Asp Val Leu Ala Lys Phe Trp Asp Glu
                485
```

This protein is encoded by a DNA molecule having a nucleotide sequence according to SEQ ID No: 3 as follows:

```
atgggtttgt tcgggatgat gaagtttgcc cacacacacc atctggtcaa gcgccggggc 60
cttggggccc cggccgggta cttcaccccc attgccgtgg acctgtggaa cgtcatgtac 120
acgttggtgg tcaaatatca gcgccgatac cccagttacg accgcgaggc cattacgcta 180
cactgcctct gtcgcttatt aaaggtgttt acccaaaagt ccctttccc catcttcgtt 240
```

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accgatcgcg gggtcaattg tatggagccg gttgtgtttg gagccaaggc catcctggcc 300
cgcacgacgg cccagtgccg gacggacgag gaggccagtg acgtggacgc ctctccaccg 360
ccttccccca tcaccgactc cagacccagc tctgcctttt ccaacatgcg ccggcgcggc 420
acctctctgg cctcggggac ccgggggacg gccgggtccg gagccgcgct gccgtccgcc 480
gcgccctcga agccggccct gcgtctggcg catctgttct gtattcgcgt tctccgggcc 540
ctggggtacg cctacattaa ctcgggtcag ctggaggcgg acgatgcctg cgccaacctc 600
tatcacacca acacggtcgc gtacgtgtac accacggaca ctgacctcct gttgatgggc 660
tgtgatattg tgttggatat tagcgcctgc tacattccca cgatcaactg tcgcgatata 720
ctaaagtact ttaagatgag ctacccccag ttcctggcct ctttgtccgc tgccacaccg 780
acctccatcc caataacacc tacgcctccg tggaggatgt gctgcgcgaa tgtcactgga 840
ccccccgag tcgctctcag acccggcggg ccatccgccg ggaacacacc agctcgcgct 900
ccacggaaac caggcccct ctgccgccgg ccgccggcgg caccgagacg cgcgtctcgt 960
ggaccgaaat tctaacccaa cagatcgccg gcggatacga agacgacgag gacctccccc 1020
tggatccccg ggacgttacc gggggccacc ccggccccag gtcgtcctcc tcggagatac 1080
tcaccccgcc cgagctcgtc caggtcccga acgcgcagct gctggaagag caccgcagtt 1140
atgtggccaa cccgcgacgc cacgtcatcc acgacgcccc agagtccctg gactggctcc 1200
ccgatcccat gaccatcacc gagctggtgg aacaccgcta cattaagtac gtcatatcgc 1260
ttatcggccc caaggagcgg gggccgtgga ctcttctgaa acgcctgcct atctaccagg 1320
acatccgcga cgaaaacctg gcgcgatcta tcgtgacccg gcatatcacg gcccctgata 1380
tcgccgacag gtttctggag cagttgcgga cccaggcccc cccacccgcg ttctacaagg 1440
acgtcctggc caaattctgg gacgagtag                                  1469
```

The amino acid and encoding nucleotide sequences of human HSV-1 vhs are reported at Genbank Accession Nos. CAA96525 and Z72338, which are hereby incorporated by reference in their entirety. The above-listed nucleotide sequence corresponds to nt 1287-2756 of SEQ ID No: 1.

Other suitable vhs proteins include human herpesvirus 2 vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. AAC58447 and AF007816, which are hereby incorporated by reference in their entirety; human herpesvirus 3 vhs protein, whose amino acid and sequence is reported as Genbank Accession No. P09275, which is hereby incorporated by reference in its entirety; bovine herpesvirus 1 vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. CAA90927 and Z54206, which are hereby incorporated by reference in their entirety; bovine herpesvirus 1.1 vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. NP_045317 and NC_001847, which are hereby incorporated by reference in their entirety; gallid herpesvirus 1 vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. AAD56213 and AF168792, which are hereby incorporated by reference in their entirety; gallid herpesvirus 2 vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. AAA80558 and L40429, which

are hereby incorporated by reference in their entirety; suid herpesvirus 1 vhs protein, whose amino acid and sequence is reported as Genbank Accession No. P36314, which is hereby incorporated by reference in its entirety; baboon herpesvirus 2 vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. AAG01880 and AF294581, which are hereby incorporated by reference in their entirety; pseudorabies virus vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. AAB25948 and S57917, which are hereby incorporated by reference in their entirety; cercopithecine herpesvirus 7 vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. NP_077432 and NC_002686, which are hereby incorporated by reference in their entirety; meleagrid herpesvirus 1 vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. NP_073335 and NC_002641, which are hereby incorporated by reference in their entirety; equine herpesvirus 1 vhs protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. NP_041028 and NC_001491, which are hereby incorporated by reference in their entirety; and equine herpesvirus 4 vhs protein, whose amino acid sequence is reported as Genbank Accession No. T42562, which is hereby incorporated by reference in its entirety.

According to one approach, the vhs vector includes a DNA molecule encoding the HSV virion host shutoff protein operatively coupled to its native transcriptional control elements. A vector of this type is prepared by excising an approximately 4.3 kb *Hpa*I/*Hind*III restriction fragment from the previously reported cosmid56 (Cunningham and Davison, "A cosmid-based system for construction mutants of herpes simplex type 1," Virology, **197**:116-124 (1993), which is hereby incorporated by reference in its entirety) and cloning the fragment into pBSKSII (Stratagene, Inc.) to create pBSKS(vhs). A map of pBSKS(vhs) is illustrated in Figure 4A. The 4.3 kb fragment includes nts 89658-93923 (complement) of the HSV-1 genome (SEQ ID No: 1, see Figures 4B-C), as reported at Genbank Accession No. X14112, which is hereby incorporated by reference in its entirety.

Optionally, the host cell which is co-transfected also expresses a suitable VP16 tegument protein. This can be achieved either by (a) transfecting the

host cell prior to the co-transfection step with a vector encoding the VP16 protein, or (b) co-transfecting a host cell which stably expresses the VP16 protein.

One suitable VP16 protein is the HSV-1 VP16 protein, which is characterized by an amino acid sequence according to SEQ ID No: 4 as follows:

```
Met Asp Leu Leu Val Asp Glu Leu Phe Ala Asp Met Asn Ala Asp Gly
  1               5                  10                  15

Ala Ser Pro Pro Pro Pro Arg Pro Ala Gly Gly Pro Lys Asn Thr Pro
             20                  25                  30

Ala Ala Pro Pro Leu Tyr Ala Thr Gly Arg Leu Ser Gln Ala Gln Leu
          35                  40                  45

Met Pro Ser Pro Pro Met Pro Val Pro Pro Ala Ala Leu Phe Asn Arg
      50                  55                  60

Leu Leu Asp Asp Leu Gly Phe Ser Ala Gly Pro Ala Leu Cys Thr Met
 65                  70                  75                  80

Leu Asp Thr Trp Asn Glu Asp Leu Phe Ser Ala Leu Pro Thr Asn Ala
                 85                  90                  95

Asp Leu Tyr Arg Glu Cys Lys Phe Leu Ser Thr Leu Pro Ser Asp Val
            100                 105                 110

Val Glu Trp Gly Asp Ala Tyr Val Pro Glu Arg Thr Gln Ile Asp Ile
        115                 120                 125

Arg Ala His Gly Asp Val Ala Phe Pro Thr Leu Pro Ala Thr Arg Asp
    130                 135                 140

Gly Leu Gly Leu Tyr Tyr Glu Ala Leu Ser Arg Phe Phe His Ala Glu
145                 150                 155                 160

Leu Arg Ala Arg Glu Glu Ser Tyr Arg Thr Val Leu Ala Asn Phe Cys
                165                 170                 175

Ser Ala Leu Tyr Arg Tyr Leu Arg Ala Ser Val Arg Gln Leu His Arg
            180                 185                 190

Gln Ala His Met Arg Gly Arg Asp Arg Asp Leu Gly Glu Met Leu Arg
        195                 200                 205

Ala Thr Ile Ala Asp Arg Tyr Tyr Arg Glu Thr Ala Arg Leu Ala Arg
    210                 215                 220

Val Leu Phe Leu His Leu Tyr Leu Phe Leu Thr Arg Glu Ile Leu Trp
225                 230                 235                 240

Ala Ala Tyr Ala Glu Gln Met Met Arg Pro Asp Leu Phe Asp Cys Leu
                245                 250                 255

Cys Cys Asp Leu Glu Ser Trp Arg Gln Leu Ala Gly Leu Phe Gln Pro
            260                 265                 270
```

```
Phe Met Phe Val Asn Gly Ala Leu Thr Val Arg Gly Val Pro Ile Glu
        275                 280                 285

Ala Arg Arg Leu Arg Glu Leu Asn His Ile Arg Glu His Leu Asn Leu
    290                 295                 300

Pro Leu Val Arg Ser Ala Ala Thr Glu Glu Pro Gly Ala Pro Leu Thr
305                 310                 315                 320

Thr Pro Pro Thr Leu His Gly Asn Gln Ala Arg Ala Ser Gly Tyr Phe
                325                 330                 335

Met Val Leu Ile Arg Ala Lys Leu Asp Ser Tyr Ser Ser Phe Thr Thr
            340                 345                 350

Ser Pro Ser Glu Ala Val Met Arg Glu His Ala Tyr Ser Arg Ala Arg
        355                 360                 365

Thr Lys Asn Asn Tyr Gly Ser Thr Ile Glu Gly Leu Leu Asp Leu Pro
    370                 375                 380

Asp Asp Asp Ala Pro Glu Glu Ala Gly Leu Ala Ala Pro Arg Leu Ser
385                 390                 395                 400

Phe Leu Pro Ala Gly His Thr Arg Arg Leu Ser Thr Ala Pro Pro Thr
                405                 410                 415

Asp Val Ser Leu Gly Asp Glu Leu His Leu Asp Gly Glu Asp Val Ala
            420                 425                 430

Met Ala His Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly
        435                 440                 445

Asp Gly Asp Ser Pro Gly Pro Gly Phe Thr Pro His Asp Ser Ala Pro
    450                 455                 460

Tyr Gly Ala Leu Asp Met Ala Asp Phe Glu Phe Glu Gln Met Phe Thr
465                 470                 475                 480

Asp Ala Leu Gly Ile Asp Glu Tyr Gly Gly
                485                 490
```

The DNA molecule encoding HSV-1 vp16 has a nucleotide sequence according to SEQ ID No: 5 as follows:

```
atggacctct tggtcgacga gctgtttgcc gacatgaacg cggacggcgc ttcgccaccg 60
ccccccgccc cggccggggg tcccaaaaac accccggcgg ccccccccgct gtacgcaacg 120
gggcgcctga gccaggccca gctcatgccc tccccaccca tgcccgtccc ccccgccgcc 180
ctctttaacc gtctcctcga cgacttgggc tttagcgcgg gccccgcgct atgtaccatg 240
ctcgatacct ggaacgagga tctgttttcg gcgctaccga ccaacgccga cctgtaccgg 300
gagtgtaaat tcctatcaac gctgcccagc gatgtggtgg aatgggggga cgcgtacgtc 360
cccgaacgca cccaaatcga cattcgcgcc cacggcgacg tggccttccc tacgcttccg 420
gccacccgcg acggcctcgg gctctactac gaagcgctct ctcgtttctt ccacgccgag 480
ctacgggcgc gggaggagag ctatcgaacc gtgttggcca acttctgctc ggccctgtac 540
cggtacctgc gcgccagcgt ccggcagctg caccgccagg cgcacatgcg cggacgcgat 600
```

```
cgcgacctgg gagaaatgct gcgcgccacg atcgcggaca ggtactaccg agagaccgct 660
cgtctggcgc gtgttttgtt tttgcatttg tatctatttt tgacccgcga gatcctatgg 720
gccgcgtacg ccgagcagat gatgcggccc gacctgtttg actgcctctg ttgcgacctg 780
gagagctggc gtcagttggc gggtctgttc cagcccttca tgttcgtcaa cggagcgctc 840
accgtccggg gagtgccaat cgaggcccgc cggctgcggg agctaaacca cattcgcgag 900
caccttaacc tcccgctggt gcgcagcgcg gctacggagg agccaggggc gccgttgacg 960
acccctccca ccctgcatgg caaccaggcc cgcgcctctg ggtactttat ggtgttgatt 1020
cgggcgaagt tggactcgta ttccagcttc acgacctcgc cctccgaggc ggtcatgcgg 1080
gaacacgcgt acagccgcgc gcgtacgaaa aacaattacg ggtctaccat cgagggcctg 1140
ctcgatctcc cggacgacga cgcccccgaa gaggcggggc tggcggctcc gcgcctgtcc 1200
tttctccccg cgggacacac gcgcagactg tcgacggccc ccccgaccga tgtcagcctg 1260
ggggacgagc tccacttaga cggcgaggac gtggcgatgg cgcatgccga cgcgctagac 1320
gatttcgatc tggacatgtt gggggacggg gattccccgg ggccgggatt tacccccac 1380
gactccgccc cctacggcgc tctggatatg gccgacttcg agtttgagca gatgtttacc 1440
gatgcccttg gaattgacga gtacggtggg tag                              1473
```

The amino acid and encoding nucleotide sequence of human HSV-1 VP16 are reported, respectively, as Genbank Accession Nos. CAA32304 and X14112, which are hereby incorporated by reference in their entirety.

Other suitable VP16 proteins include human herpesvirus 2 VP16 protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. NP_044518 and NC_001798, which are hereby incorporated by reference in their entirety; bovine herpesvirus 1 VP16 protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. CAA90922 and Z54206, which are hereby incorporated by reference in their entirety; bovine herpesvirus 1.1 VP16 protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. NP_045311 and NC_001847, which are hereby incorporated by reference in their entirety; gallid herpesvirus 1 VP16 protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. BAA32584 and AB012572, which are hereby incorporated by reference in their entirety; gallid herpesvirus 2 VP16 protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. NP_057810 and NC_002229, which are hereby incorporated by reference in their entirety; meleagrid herpesvirus 1 VP16 protein, whose amino acid and encoding nucleotide sequences are reported, respectively, as Genbank Accession Nos. AAG30088 and AF282130, which are hereby incorporated by reference in their entirety; and equine herpesvirus 4 VP16 protein, whose amino acid and encoding

nucleotide sequences are reported as Genbank Accession Nos. NP_045229 and NC_001844, which are hereby incorporated by reference in their entirety.

When performing an initial transfection step prior to co-transfection, the transfection with a vector encoding the VP16 protein can be carried out at least about 1 hour before the co-transfection step, more preferably at least about 4 hours before, and most preferably at least about 12 hours before. Maximal amplicon particle titers have been achieved following transfection of host cells (with VP16) about 24 hours prior to the co-transfection step described below. When prior transfection of the host cell is carried out, a preferred vector encoding the HSV-1 VP16 protein is vector $pGRE_5vp16$, whose structure is illustrated in Figure 5.

In host cells transiently expressing VP16, the plasmid encoding VP16 is lost in up to about 50% of the cells per doubling of the cell population.

Stable expression of VP16 can be achieved either using a stable plasmid which is copied and partitioned among dividing host cells with acceptable fidelity or by integration of the VP16 into the host cell genome. Plasmids which are stable in *in vitro* cell lines are known in the art and can be used to introduce *UL48* thereon. Also, integration can be carried out according to known procedures.

Preparation of HSV amplicon particles can be carried out by co-transfecting a suitable host cell with (i) the amplicon vector, (ii) either the set of cosmid vectors or BAC, and (iii) the vhs expression vector. Basically, the various vectors are introduced into a single medium (e.g., Opti-MEM available from Gibco-BRL, Bethesda, MD) within a container (e.g., sterile polypropylene tube), forming a DNA mix. The weight ratio of BAC:amplicon vector is between about 1-10:1, preferably about 5-10:1, and the weight ratio of 5 cosmid set (in total):amplicon vector is between about 1-10:1, preferably about 2-7:1. The DNA mix is later introduced into a container (with Lipofectamine reagent) which has been seeded with the host cells to be co-transfected. Thereafter, the transfection mix is diluted with an equal volume of a selection medium (e.g., DMEM plus 20% FBS, 2% penicillin/streptomycin, and 2mM hexamethylene bis-acetamide (HMBA)) and incubated for several days. Virion particles are released from the host cells by sonication and purified from host cell protein/membrane components via ultracentrifugation.

When prior transfection is effected, allowing the host cells to express HSV-1 VP16 prior to co-transfection as described above, the cells plated for packaging were first allowed to adhere to a culture dish and subsequently transfected with $pGRE_5vp16$ using Lipofectamine reagent. Following suitable incubation, the transfection mix was removed, complete medium (e.g., DMEM plus 10% FBS, 1% penicillin/streptomycin) was added, and the cultures were incubated at 37°C until the packaging co-transfection step described above.

Suitable host cells which can be co-transfected for preparation of HSV amplicon particles are eukaryotic cells, preferably mammalian cells. Exemplary host cells include, without limitation, BHK cells, NIH 3T3 cells, 2-2 cells, 293 cells, and RR1 cells.

When the HSV amplicon particles are harvested from the host cell medium, the amplicon particles are substantially pure (i.e., free of any other virion particles) and present at a concentration of greater than about 1×10^6 particles per milliliter. To further enhance the use of the amplicon particles, the resulting stock can also be concentrated, which affords a stock of isolated HSV amplicon particles at a concentration of at least about 1×10^7 particles per milliliter.

The resulting amplicon particles produced according to the present invention, i.e., in the presence of vhs and, optionally VP16, both of which can be expressed in host cells prior to packaging, are substantially different in kind from the virion particles which can be prepared using known helper virus methods (see Examples 1 and 4).

The concentrated stock of HSV amplicon particles is effectively a composition of the HSV amplicon particles in a suitable carrier. Alternatively, the HSV amplicon particles may also be administered in injectable dosages by dissolution or suspension of these materials in a physiologically acceptable diluent with a pharmaceutical carrier. Such carriers include sterile liquids, such as water and oils, with or without the addition of a surfactant and other pharmaceutically and physiologically acceptable carriers, including adjuvants, excipients or stabilizers. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols, such as propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions.

For use as aerosols, the HSV amplicon particles, in solution or suspension, may be packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The materials of the present invention also may be administered in a non-pressurized form such as in a nebulizer or atomizer.

The pharmaceutical composition is preferably in liquid form, such as a solution, suspension, or emulsion. Typically, the composition will contain at least about 1×10^7 amplicon particles/ml, together with the carrier, excipient, stabilizer, etc.

A further aspect of the present invention relates to a system for preparing HSV amplicon particles. The system includes (i) an empty amplicon vector as described above, which includes an HSV origin of replication, an HSV cleavage/packaging signal, and a transgene insertion site (at which a transgene may be inserted, as described above), (ii) one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals, and (iii) a vhs expression vector encoding a virion host shutoff protein. The vhs expression vector is of the type described above. The system is characterized as being able to produce HSV amplicon particles of the present invention when the system is introduced (i.e., co-transfected) into a suitable host cell. The system may further include, as described above, a host cell which stably expresses an HSV VP16 protein and/or a vector encoding the HSV VP16 protein.

Yet another aspect of the present invention relates to a kit for preparing HSV amplicon particles of the present invention. The kits includes: (i) an amplicon vector comprising an HSV origin of replication, an HSV cleavage/packaging signal, and a transgene insertion site (at which a transgene may be inserted, as described above), (ii) one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals, (iii) a vhs expression vector encoding an virion host shutoff protein, (iv) a population of host cells susceptible to transfection by the amplicon vector, the vhs expression vector, and the one or more vectors, and (v) directions for transfecting the host cells under conditions to produce HSV amplicon particles. The vhs expression vector is of the type described above. The kit may further include, as described above, a host cell which stably expresses an HSV VP16 protein and/or a vector encoding the HSV VP16 protein.

Yet another aspect of the present invention relates generally to a method of expressing a therapeutic gene product in a patient using the HSV amplicon particles of the present invention which contain a transgene encoding a therapeutic gene product. Basically, this method is carried out by providing such HSV amplicon particles and exposing patient cells to the HSV amplicon particles under conditions effective for infective transformation of the cells, wherein the therapeutic transgene product is expressed *in vivo* in transformed cells. As noted below, transformation of the patient cells can be carried out *in vivo* or *ex vivo*.

HSV-1 has a wide host range and infects many cell types in mammals and birds (including chickens, rats, mice, monkeys, humans) (Spear et al., DNA Tumor Viruses, pp. 615-746, Tooze, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1981), which is hereby incorporated by reference in its entirety). HSV-1 can lytically infect a wide variety of cells including, e.g., neurons, fibroblasts, and macrophages. In addition, HSV-1 infects post-mitotic neurons in adult animals and can be maintained indefinitely in a latent state (Stevens, Curr. Topics in Microbiol. and Immunol. **70**:31-50 (1975), which is hereby incorporated by reference in its entirety). Two lines of evidence suggest that HSV-1 can infect most, if not all, kinds of neurons in the central nervous system. First, following inoculation of HSV-1 in the periphery, a burst of virus production ascends the neuroaxis, initially in the sensory or motor neurons innervating the site of inoculation, then in the spinal cord, brain stem, cerebellum, and cerebral cortex (Koprowski, In Persistent Viruses, pp. 691-699, Stevens, ed., Academic Press, New York, New York (1978), which is hereby incorporated by reference in its entirety). Second, attempts to mimic HSV-1 latency in tissue culture with different preparations of neurons have required high temperature, DNA synthesis inhibitors, and antisera directed against HSV-1 virions to prevent lytic infection for spreading to all neurons (Wigdahl et al., Proc. Natl. Acad. Sci. USA **81**:6217-6201 (1984), which is hereby incorporated by reference in its entirety).

Because HSV-1 infects a wide range of animals, the HSV amplicon particles of the present invention can be used on a wide variety of mammals and birds. Preferably, the HSV amplicon particles are used on mammals, most preferably humans, to effect expression of the therapeutic transgene product. Thus, as used herein, patient refers generally to mammals and birds, as well as humans specifically.

When exposing the patient cells to the HSV amplicon particles, an *in vivo* route of delivery is performed by administering the HSV amplicon particles directly to the patient cells which are to be transformed. The administering can be achieved in a manner which is suitable to effect delivery and subsequent patient cell transformation, including, without limitation, intraparenchymal, intramuscular, intravenous, intracerebroventricular, subcutaneous, or intramucosal delivery.

Alternatively, an *ex vivo* route of delivery is performed by providing patient cells (either removed from the patient or obtained from a donor), exposing the cells *ex vivo* to the HSV amplicon particles, and then introducing the transformed cells into the patient. Stem cells, embryonic or progenitor, can be effectively transformed and then introduced into the patient at a desired location. For non-motile transformed cells, such cells are preferably administered to the patient at the site where the cells are intended to reside. For actively or passively motile transformed cells, such cells may be administered in a manner which is effective to deliver the transformed cells into the patient. Suitable delivery routes include, without limitation, intraparenchymal, intramuscular, intravenous, intracerebroventricular, subcutaneous, or intramucosal delivery.

Still another aspect of the present invention relates to a method of treating a neurological disease or disorder using the HSV amplicon particles of the present invention which include a transgene encoding a therapeutic transgene product. Basically, this method is carried out by providing such HSV amplicon particles and exposing patient neural or pre-neural cells to the HSV amplicon particles under conditions effective for infective transformation of neural or pre-neural cells of the patient, wherein the therapeutic transgene product is expressed *in vivo* by the neural or pre-neural cells, thereby treating the neurological disease or disorder.

As noted above, transformation can be effected either *in vivo* or *ex vivo* (i.e., using differentiated neural cells, neural stem cells, or embryonic stem cells which differentiate into neural cells). A preferred *in vivo* route of delivery is administering the HSV amplicon particles directly to neural cells which are to be treated using, e.g., the delivery routes listed above.

Neuronal diseases or disorders which can be treated include lysosomal storage diseases (e.g., by expressing MPS 1-VIII, hexoaminidase A/B, etc.), Lesch-Nyhan syndrome (e.g., by expressing HPRT), amyloid polyneuropathy (e.g., by

expressing ß-amyloid converting enzyme (BACE) or amyloid antisense), Alzheimer's Disease (e.g., by expressing NGF, ChAT, BACE, etc.), retinoblastoma (e.g., by expressing pRB), Duchenne's muscular dystrophy (e.g., by expressing Dystrophin), Parkinson's Disease (e.g., by expressing GDNF, Bcl-2, TH, AADC, VMAT, antisense to mutant alpha-synuclein, etc.), Diffuse Lewy Body disease (e.g., by expressing heat shock proteins, parkin, or antisense or RNAi to alpha-synuclein), stroke (e.g., by expressing Bcl-2, HIF-DN, BMP7, GDNF, other growth factors), brain tumor (e.g., by expressing angiostatin, antisense VEGF, antisense or ribozyme to EGF or scatter factor, pro-apoptotic proteins), epilepsy (e.g., by expressing GAD65, GAD67, pro-apoptotic proteins into focus), or arteriovascular malformation (e.g., by expressing proapoptotic proteins).

Likewise, the HSV amplicon particles of the present invention which include a transgene encoding a therapeutic transgene product can also be used according to a method of inhibiting development of a neurological disease or disorder. Basically, this method is carried out by providing such HSV amplicon particles and exposing neural or pre-neural cells of the patient who is susceptible to development of a neurological disease or disorder to the HSV amplicon particles under conditions effective for infective transformation of the neural or pre-neural cells, wherein the therapeutic transgene product is expressed *in vivo* by the neural or pre-neural cells, thereby inhibiting development of the neurological disease or disorder.

As noted above, transformation can be effected either *in vivo* or *ex vivo* (i.e., using differentiated neural cells, neural stem cells, or embryonic stem cells which differentiate into neural cells). A preferred *in vivo* route of delivery is administering the HSV amplicon particles directly to the neural cells which are to be treated using, e.g., the delivery routes listed above. The neuronal disease or disorder whose development can be inhibited, and the therapeutic transgene product associated therewith, are those which are listed above by way of example.

In addition to the foregoing uses described, the HSV amplicon particles of the present invention can also be used for delivery of other therapeutic transgenes as reported previously in the literature (i.e., using other vectors or HSV-derived vectors prepared according to helper-virus procedures or previously reported helper virus-free procedures). By way of example, Kutubuddin et al., "Eradication of Pre-Established Lymphoma Using Herpes Simplex Virus Amplicon Vectors," Blood

93(2):643-654 (1999), which is hereby incorporated by reference in its entirety, reports on the use of helper virus-prepared HSV amplicon particles which transduce CD80 or RANTES, eliciting a protective immune response to pre-established lymphoma and generating tumor-specific cytotoxic T-cells immunity and immunologic memory.

EXAMPLES

The following examples are provided to illustrate an embodiment of the present invention but is by no means intended to limit its scope.

Materials & Methods

Cell Culture

Baby hamster kidney (BHK) cells were maintained as described before (Lu and Federoff, "Herpes simplex virus type 1 amplicon vectors with glucocorticoid-inducible gene expression," Hum. Gene Ther. **6**:421-430 (1995), which is hereby incorporated by reference in its entirety). The NIH-3T3 mouse fibroblast cell line was originally obtained from American Type Culture Collection and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin.

Plasmid Construction

The HSVPrPUC/CMVegfp amplicon plasmid was constructed by cloning the 0.8-kb cytomegalovirus (CMV) immediate early promoter and 0.7-kb enhanced green fluorescent protein cDNA (Clontech, Inc.) into the BamHI restriction enzyme site of the pHSVPrPUC amplicon vector.

A 3.5 kb Hpa I/Hind III fragment encompassing the UL41 (*vhs*) open reading frame and its 5' and 3' transcriptional regulatory elements was removed from *cos56* (Cunningham and Davison, "A cosmid-based system for construction mutants of herpes simplex type 1," Virology, **197**:116-124 (1993), which is hereby incorporated by reference in its entirety) and cloned into pBSKSII (Stratagene, Inc.) to create pBSKS(vhs).

For construction of $pGRE_5vp16$, the VP16 coding sequence was amplified by PCR from pBAC-V2 using gene-specific oligonucleotides that possess

EcoRI and HindIII restriction enzyme sequences that facilitates cloning into the pGRE$_5$-2 vector (Mader and White, "A steroid-inducible promoter for the controlled overexpression of cloned genes in eukaryotic cells," Proc. Natl. Acad. Sci. USA, **90**:5603-5607 (1993), which is hereby incorporated by reference in its entirety). The oligonucleotide possessing the EcoRI site has a nucleotide sequence according to SEQ ID No: 6 as follows:

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cggaattccg caggttttgt aatgtatgtg ctcgt                    35
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The oligonucleotide possessing the HindIII site has a nucleotide sequence according to SEQ ID No: 7 as follows:

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ctccgaagct taagcccgat atcgtctttc ccgtatca                 38
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Helper virus-free amplicon packaging

On the day prior to transfection, 2x10^6 BHK cells were seeded on a 60-mm culture dish and incubated overnight at 37°C. For cosmid-based packaging: The day of transfection, 250 µl Opti-MEM (Gibco-BRL, Bethesda, MD), 0.4 µg of each of the five cosmid DNAs and 0.5 µg amplicon vector DNA with or without varying amounts of pBSKS(vhs) plasmid DNA were combined in a sterile polypropylene tube (Fraefel et al., "Helper virus-free transfer of herpes simplex virus type 1 plasmid vectors into neural cells," J. Virol., **70**:7190-7197 (1996), which is hereby incorporated by reference in its entirety). For BAC-based packaging: 250 µl Opti-MEM (Gibco-BRL, Bethesda, MD), 3.5 µg of pBAC-V2 DNA and 0.5 µg amplicon vector DNA with or without varying amounts of pBSKS(vhs) plasmid DNA were combined in a sterile polypropylene tube (Stavropoulos and Strathdee, "An enhanced packaging system for helper-dependent herpes simplex virus vectors," J. Virol., **72**:7137-43 (1998), which is hereby incorporated by reference in its entirety). The protocol for both cosmid- and BAC-based packaging was identical from the following step forward. Ten microliters of Lipofectamine Plus Reagent (Gibco-BRL) were added over a 30-second period to the DNA mix and allowed to incubate at RT for 20 min. In a separate tube, 15 µl Lipofectamine (Gibco-BRL) were mixed with 250 µl Opti-MEM. Following the 20-min incubation, the contents of the two tubes were

combined over a 1-min period, and incubated for an additional 20 min at RT. During the second incubation, the medium in the seeded 60-mm dish was removed and replaced with 2 ml Opti-MEM. The transfection mix was added to the flask and allowed to incubate at 37°C for 5 hrs. The transfection mix was then diluted with an equal volume of DMEM plus 20% FBS, 2% penicillin/streptomycin, and 2mM hexamethylene bis-acetamide (HMBA), and incubated overnight at 34°C. The following day, medium was removed and replaced with DMEM plus 10% FBS, 1% penicillin/streptomycin, and 2mM HMBA. The packaging flask was incubated an additional 3 days and virus harvested and stored at –80°C until purification. Viral preparations were subsequently thawed, sonicated, and clarified by centrifugation (3000 x g, 20 min.). Viral samples were stored at –80°C until use. For packaging experiments examining the effect of VP16 on amplicon titers, the cells plated for packaging were first allowed to adhere to the 60-mm culture dish for 5 hours and subsequently transfected with $pGRE_5vp16$ using the Lipofectamine reagent as described above. Following a 5-hr incubation, the transfection mix was removed, complete medium (DMEM plus 10% FBS, 1% penicillin/streptomycin) was added, and the cultures were incubated at 37°C until the packaging co-transfection step the subsequent day.

Viral Titering

Amplicon titers were determined by counting the number of cells expressing enhanced green fluorescent protein (HSVPrPUC/CMVegfp amplicon) or β-galactosidase (HSVlac amplicon). Briefly, 10 µl of concentrated amplicon stock was incubated with confluent monolayers ($2x10^5$ expressing particles) of NIH 3T3 cells plated on glass coverslips. Following a 48-hr incubation, cells were either fixed with 4% paraformaldehyde for 15 min at RT and mounted in Moiwol for fluorescence microscopy (eGFP visualization), or fixed with 1% glutaraldehyde and processed for X-gal histochemistry to detect the *lacZ* transgene product. Fluorescent or X-gal-stained cells were enumerated, expression titer calculated, and represented as either green-forming units per ml (gfu/ml) or blue-forming units per ml (bfu/ml), respectively.

TaqMan Quantitative PCR System

To isolate total DNA for quantitation of amplicon genomes in packaged stocks, virions were lysed in 100 mM potassium phosphate pH 7.8 and

0.2% Triton X-100. Two micrograms of genomic carrier DNA was added to each sample. An equal volume of 2X Digestion Buffer (0.2 M NaCl, 20 mM Tris-Cl pH 8, 50 mM EDTA, 0.5% SDS, 0.2 mg/ml proteinase K) was added to the lysate and the sample was incubated at 56°C for 4 hrs. Samples were processed further by one phenol:chloroform, one chloroform extraction, and a final ethanol precipitation. Total DNA was quantitated and 50 ng of DNA was analyzed in a PE7700 quantitative PCR reaction using a designed *lacZ*-specific primer/probe combination multiplexed with an 18S rRNA-specific primer/probe set. The *lacZ* probe sequence (SEQ ID No: 8) was as follows:

6FAM-accccgtacg tcttcccgag cg-TAMRA 22

where 6FAM is a (6-carboxyfluorescein) conjugated dye and TAMRA is a (6-carboxytetramethylrhodamine) conjugated quencher. The *lacZ* sense primer sequence (SEQ ID No: 9) was as follows:

gggatctgcc attgtcagac at 22

The *lacZ* antisense primer sequence (SEQ ID No: 10) was as follows:

tggtgtgggc cataattcaa 20

The 18S rRNA probe sequence (SEQ ID No: 11) was as follows:

JOE-tgctggcacc agacttgccc tc-TAMRA 22

where JOE is a (6-carboxy-4',5'-dichloro-2', 7'-dimethoxyfluorescein) conjugated dye. The 18S sense primer sequence (SEQ ID No: 12) was as follows:

cggctaccac atccaaggaa 20

The 18S antisense primer sequence (SEQ ID No: 13) was as follows:

gctggaatta ccgcggct 18

Each 25-µl PCR sample contained 2.5 µl (50 ng) of purified DNA, 900 nM of each primer, 50 nM of each probe, and 12.5 µl of 2X Perkin-Elmer Master

Mix. Following a 2-min 50°C incubation and 2-min 95°C denaturation step, the samples were subjected to 40 cycles of 95°C for 15 sec. and 60°C for 1 min. Fluorescent intensity of each sample was detected automatically during the cycles by the Perkin-Elmer Applied Biosystem Sequence Detector 7700 machine. Each PCR run included the following: no-template control samples, positive control samples consisting of either amplicon DNA (for *lacZ*) or cellular genomic DNA (for 18S rRNA), and standard curve dilution series (for *lacZ* and 18S). Following the PCR run, "real-time" data were analyzed using Perkin-Elmer Sequence Detector Software version 1.6.3 and the standard curves. Precise quantities of starting template were determined for each titering sample and results were expressed as numbers of vector genomes per ml of original viral stock.

Western blot analysis

BHK cell monolayers (2×10^6 cells) transfected with varying packaging components were lysed with RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% DOC, 0.5% SDS, and 50 mM Tris-Cl, pH 8). Equal amounts of protein were electrophoretically separated on a 10% SDS-PAGE gel and transferred to a PVDF membrane. The resultant blot was incubated with an anti-VP16 monoclonal antibody (Chemicon, Inc.), and specific VP16 immunoreactive band visualized using an alkaline phosphatase-based chemiluminescent detection kit (ECL).

Stereotactic injections

Mice were anesthetized with Avertin at a dose of 0.6 ml per 25 g body weight. After positioning in an ASI murine stereotactic apparatus, the skull was exposed via a midline incision, and burr holes were drilled over the following coordinates (bregma, +0.5 mm; lateral - 2.0 mm; and deep, -3.0 mm) to target infections to the striatum. A 33 GA steel needle was gradually advanced to the desired depth, and 3 µl of HSVPrPUC/CMVegfp virus was infused via a microprocessor-controlled pump over 10 minutes (UltraMicroPump, World Precision Instruments, Sarasota Springs, Fla.). The injector unit was mounted on a precision small animal stereotaxic frame (ASI Instruments, Warren, MI) micromanipulator at a 90^o angle using a mount for the injector. Viral injections were performed at a constant rate of 300 nl/min. The needle was removed slowly over an additional 10-minute period.

Tissue preparation and GFP visualization

Infected mice were anesthetized four days later, a catheter was placed into the left ventricle, and intracardiac perfusion was initiated with 10 ml of heparinized saline (5,000 U/L saline) followed by 60 ml of chilled 4% PFA. Brains were extracted and postfixed for 1-2 hours in 4% PFA at 4°C. Subsequently, brains were cryoprotected in a series of sucrose solutions with a final solution consisting of a 30% sucrose concentration (w/v) in PBS. Forty micron serial sections were cut on a sliding microtome (Micron/Zeiss, Thornwood, NY) and stored in a cryoprotective solution (30% sucrose (w/v), 30% ethylene glycol in 0.1 M phosphate buffer (pH 7.2)) at -20°C until processed for GFP visualization. Sections were placed into Costar net wells (VWR, Springfield, NJ) and incubated for 2 hrs in 0.1 M Tris buffered saline (TBS) (pH=7.6). Upon removal of cryoprotectant, two additional 10 min washes in 0.1 M TBS with 0.25% Triton X-100 (Sigma, St. Louis, MO) were performed. Sections were mounted with a fine paint brush onto subbed slides, allowed to air dry, and mounted with an aqueous mounting media, Mowiol. GFP-positive cells were visualized with a fluorescent microscope (Axioskop, Zeiss, Thornwood, NY) utilizing a FITC cube (Chroma Filters, Brattleboro, VT). All images used for morphological analyses were digitally acquired with a 3-chip color CCD camera at 200x magnification (DXC-9000, Sony, Montvale, NJ).

Morphological analyses

Cell counts were performed on digital images acquired within 24 hrs of mounting. At the time of tissue processing coronal slices were stored serially in three separate compartments. All compartments were processed for cell counting and GFP(+) cell numbers reflect cell counts throughout the entire injection site. All spatial measurements were acquired using an image analysis program (Image-Pro Plus, Silver Spring, MD) at a final magnification of 200x. Every section was analyzed using identical parameters in three different planes of focus throughout the section to prevent repeated scoring of GFP(+) cells. Each field was analyzed by a computer macro to count cells based on the following criteria: object area, image intensity (fluorescent signal) and plane of focus. Only cells in which the cell body was unequivocally GFP(+) and nucleus clearly defined were counted. Every section that contained a GFP-positive cell was counted. In addition, a watershed separation technique was applied to every plane of focus in each field to delineate overlapping

cell bodies. The watershed method is an algorithm that is designed to erode objects until they disappear, then dilates them again such that they do not touch.

Example 1 - Effect of Amplicon Co-transfection with vhs Vector

To determine if introduction of vhs into the packaging scheme could increase amplicon titers and quality, a genomic segment of the UL41 gene was cloned into pBluescript and the resulting plasmid (pBSKS(vhs)) was introduced into co-transfection protocols to provide vhs *in trans*. The genomic copy of UL41 contained the transcriptional regulatory region and flanking *cis* elements believed to confer native UL41 gene expression during packaging. When pBSKS(vhs) was added to the packaging protocols for production of a β-galactosidase (*lacZ*)-expressing amplicon (HSVlac), a maximum of 10-fold enhanced amplicon expression titers was observed for both cosmid- and BAC-based strategies (Figure 6A and B, respectively). As observed previously, the expression titers for HSVlac virus produced by the BAC-based method were approximately 500- to 1000-fold higher than stocks produced using the modified cosmid set. Even though a large disparity existed between the differently prepared stocks, the effect of additionally expressed vhs on amplicon titers was analogous.

The punctate appearance of reporter gene product (pseudotransduction), a phenomenon associated with first-generation helper virus-free stocks, was drastically diminished *in vitro* when vhs was included in BAC-based packaging of an enhanced green fluorescent (GFP)-expressing virus (HSVPrPUC/CMVegfp) (Figures 7C-D). Pseudotransduction was not observed, as well, for cosmid-packaged amplicon stocks prepared in the presence of vhs.

To assess the ability of the improved amplicon stocks to mediate gene delivery *in vivo*, 3 μl of BAC-packaged HSVPrPUC/CMVegfp virus prepared in the absence or presence of pBSKS(vhs) was injected stereotactically into the striata of C57BL/6 mice. Four days following infection, animals were sacrificed and analyzed for GFP-positive cells present in the striatum (Figures 7E-F). The numbers of cells transduced by HSVPrPUC/CMVegfp prepared in the presence of vhs were significantly higher than in animals injected with stocks produced in the absence of

vhs (Figure 7G). In fact, it was difficult to definitively identify GFP-positive cells in animals transduced with vhs(-) amplicon stocks.

The mechanism by which vhs expression resulted in higher apparent amplicon titers in helper virus-free packaging could be attributed to one or several properties of vhs. The UL41 gene product is a component of the viral tegument and could be implicated in structural integrity, and its absence could account for the appearance of punctate gene product material following transduction. For example, the viral particles may be unstable as a consequence of lacking vhs. Thus, physical conditions, such as repeated freeze-thaw cycles or long-term storage, may have led to inactivation or destruction of vhs-lacking virions at a faster rate than those containing vhs.

The stability of HSVPrPUC/CMVegfp packaged via the BAC method in the presence or absence of vhs was analyzed initially with a series of incubations at typically used experimental temperatures. Viral aliquots from prepared stocks of HSVPrPUC/CMVegfp were incubated at 4, 22, or 37°C for periods up to three hours. Virus recovered at time points 0, 30, 60, 120, and 180 minutes were analyzed for their respective expression titer on NIH 3T3 cells. The rates of decline in viable amplicon particles, as judged by their ability to infect and express GFP, did not differ significantly between the vhs(+) and vhs(-) stocks (Figures 8A-C). Another condition that packaged amplicons encounter during experimental manipulation is freeze-thaw cycling. Repetitive freezing and thawing of virus stocks is known to diminish numbers of viable particles, and potentially the absence of vhs in the tegument of pBAC-V2 packaged amplicons leads to sensitivity to freeze fracture. To test this possibility, viral aliquots were exposed to a series of four freeze-thaw cycles. Following each cycle, samples were removed and titered for GFP expression on NIH 3T3 cells as described previously. At the conclusion of the fourth freeze-thaw cycle, the vhs(-) HSVPrPUC/CMVegfp stock exhibited a 10-fold diminution in expression titers as opposed to only a 2-fold decrease for vhs(+) stocks (Figure 8D). This observation suggests that not only do vhs(+) stocks have increased expression titers, but the virions are more stable when exposed to temperature extremes, as determined by repetitive freeze-thaw cycling.

Wild-type HSV virions contain multiple regulatory proteins that prepare an infected host cell for virus propagation. One of these virally encoded

regulators, which is localized to the tegument, is vhs. The *UL41* gene-encoded vhs protein exhibits an essential endoribonucleolytic cleavage activity during lytic growth that destabilizes both cellular and viral mRNA species (Smibert et al., "Identification and characterization of the virion-induced host shutoff product of herpes simplex virus gene UL41," J. Gen. Virol., **73**:467-470 (1992), which is hereby incorporated by reference in its entirety). Vhs-mediated ribonucleolytic activity appears to prefer the 5' ends of mRNAs over 3' termini, and the activity is specific for mRNA, as vhs does not act upon ribosomal RNAs (Karr and Read, "The virion host shutoff function of herpes simplex virus degrades the 5' end of a target mRNA before the 3' end," Virology, **264**:195-204 (1999), which is hereby incorporated by reference in its entirety). Vhs also serves a structural role in virus particle maturation as a component of the tegument. HSV isolates that possess disruptions in UL41 demonstrate abnormal regulation of IE gene transcription and significantly lower titers than wild-type HSV-1 (Read and Frenkel, "Herpes simplex virus mutants defective in the virion-associated shutoff of host polypeptide synthesis and exhibiting abnormal synthesis of α (immediate early) viral polypeptides," J. Virol., **46**:498-512 (1983), which is hereby incorporated by reference in its entirety), presumably due to the absence of vhs activity. Therefore, because vhs is essential for efficient production of viable wild-type HSV particles, it likely plays a similarly important role in packaging of HSV-1-derived amplicon vectors.

The term "pseudotransduction" refers to virion expression-independent transfer of biologically active vector-encoded gene product to target cells (Liu et al., "Pseudotransduction of hepatocytes by using concentrated pseudotyped vesicular stomatitis virus G glycoprotein (VSV-G)-Moloney murine leukemia virus-derived retrovirus vectors: comparison of VSV-G and amphotrophic vectors for hepatic gene transfer," J. Virol., **70**: 2497-2502 (1996); Alexander et al., "Transfer of contaminants in adeno-associated virus vector stocks can mimic transduction and lead to artifactual results," Hum. Gene Ther., **8**:1911-1920 (1997); Yu et al., "High efficiency in vitro gene transfer into vascular tissues using a pseudotyped retroviral vector without pseudotransduction," Gene Ther., **6**:1876-1883 (1999), which are hereby incorporated by reference in their entirety). This phenomenon was originally described with retrovirus and adeno-associated virus vector stocks and was shown to result in an overestimation of gene transfer efficiencies. β-galactosidase and alkaline phosphatase

are two commonly expressed reporter proteins that have been implicated in pseudotransduction, presumably due to their relatively high enzymatic stability and sensitivity of their respective detection assays (Alexander et al., "Transfer of contaminants in adeno-associated virus vector stocks can mimic transduction and lead to artifactual results," Hum. Gene Ther., **8**:1911-1920 (1997), which is hereby incorporated by reference in its entirety). Stocks of β-galactosidase-expressing HSVlac and GFP-expressing HSVPrPUC/CMVegfp exhibited high levels of pseudotransduction when packaged in the absence of vhs. Upon addition of vhs to the previously described helper virus-free packaging protocols (Fraefel et al., "Helper virus-free transfer of herpes simplex virus type 1 plasmid vectors into neural cells," J. Virol., **70**:7190-7197 (1996); Stavropoulos and Strathdee, "An enhanced packaging system for helper-dependent herpes simplex virus vectors," J. Virol., **72**:7137-43 (1998), which are hereby incorporated by reference in their entirety), a 10-fold increase in expression titers and concomitant decrease in pseudotransduction were observed *in vitro*.

Vhs-mediated enhancement of HSV amplicon packaging was even more evident when stocks were examined *in vivo*. GFP-expressing cells in animals transduced with vhs(+) stocks were several hundred-fold greater in number than in animals receiving vhs(-) stocks. This could have been due to differences in virion stability, where decreased particle stability could have led to release of co-packaged reporter gene product observed in the case of vhs(-) stocks. Additionally, the absence of vhs may have resulted in packaging of reporter gene product into particles that consist of only tegument and envelope (Rixon et al., "Assembly of enveloped tegument structures (L particles) can occur independently of virion maturation in herpes simplex virus type 1-infected cells," J. Gen. Virol., **73**:277-284 (1992), which is hereby incorporated by reference in its entirety). Release of co-packaged reporter gene product in either case could potentially activate a vigorous immune response in the CNS, resulting in much lower than expected numbers of vector-expressing cells.

Interestingly, the HSV-encoding cosmid set harbored an intact *UL41* gene locus (Cunningham and Davison, "A cosmid-based system for construction mutants of herpes simplex type 1," Virology, **197**:116-124 (1993), which is hereby incorporated by reference), while the BAC reagent that was utilized for helper virus-free packaging did not because of a disruption introduced during its initial

construction (Stavropoulos and Strathdee, "An enhanced packaging system for helper-dependent herpes simplex virus vectors," J. Virol., **72**:7137-43 (1998), which is hereby incorporated by reference). Expression of vhs via a co-transfected plasmid containing the entire *UL41* gene plus its cognate transcriptional regulatory regions resulted in pronounced increases in packaged amplicon produced via either cosmid- or BAC-based method. For BAC-based packaging, the explanation appears rather clear: vhs is not expressed due to disruption of the UL41 locus, and therefore, inclusion of a vhs expression plasmid results in a more productive packaging. In the case for cosmid-based packaging, the copy number of the co-transfected vhs-encoding plasmid greatly exceeded the number of vhs transcription units present in the cosmid set. This likely led to a more rapid accumulation of vhs during the early stages of packaging. Additionally, because the cosmid set is believed to undergo recombination of its overlapping homologous regions to produce a HSV genome-sized unit following introduction into the packaging cell, perhaps viral gene expression is delayed (Cunningham and Davison, "A cosmid-based system for construction mutants of herpes simplex type 1," Virology, **197**:116-124 (1993), which is hereby incorporated by reference). As a result, amplicon propagation cannot optimally initiate.

The resulting HSV amplicon particles were also examined by scanning electron micrography using a standard negative staining technique (Monroe and Brandt, "Rapid semiquantitative method for screening large numbers of virus samples by negative staining electron microscopy," Appl Microbiol **20**(2):259-62 (1970), which is hereby incorporated by reference in its entirety). As shown in Figure 13, the HSV amplicon particles, denoted by arrows, are substantially smaller in size than the 173 nm reference spheres and rather heterogeneous in structure. In contrast, helper virus-containing stocks are characterized by the production of HSV amplicon particles which are approximately 150 nm in size and more homogeneous in shape. Thus, the HSV amplicon particles of the present invention are physically different from previously known helper virus-prepared HSV amplicon particles.

Example 2 - Effect of VP16 Expression in Host Cells Prior to Amplicon Co-transfection

The native HSV genome enters the host cell with several viral proteins besides vhs, including the strong transcriptional activator VP16. Once within the cell, VP16 interacts with cellular transcription factors and HSV genome to initiate immediate-early gene transcription. Under helper virus-free conditions, transcriptional initiation of immediate-early gene expression from the HSV genome may not occur optimally, thus leading to lower than expected titers. To address this issue, a VP16 expression construct was introduced into packaging cells prior to cosmid/BAC, amplicon, and pBSKS(vhs) DNAs, and resultant amplicon titers were measured. To achieve regulated expression a glucocorticoid-controlled VP16 expression vector was used ($pGRE_5vp16$).

The $pGRE_5vp16$ vector was introduced into the packaging cells 24 hours prior to transfection of the regular packaging DNAs. HSVlac was packaged in the presence or absence of vhs and/or VP16 and resultant amplicon stocks were assessed for expression titer. Some packaging cultures received 100 nM dexamethasone at the time of $pGRE_5vp16$ transfection to strongly induce VP16 expression; others received no dexamethasone. Introduction of $pGRE_5vp16$ in an uninduced (basal levels) or induced state (100 nM dexamethasone) had no effect on HSVlac titers when vhs was absent from the cosmid- or BAC-based protocol (Figures 9A-B). In the presence of vhs, addition of $pGRE_5vp16$ led to either a two- or five-fold enhancement of expression titers over those of stocks packaged with only vhs (cosmid- and BAC-derived stocks, respectively; Figures 9A-B). The effect of "uninduced" $pGRE_5vp16$ on expression titers suggested that VP16 expression was occurring in the absence of dexamethasone. To demonstrate this, Western blot analysis with a VP16-specific monoclonal antibody was performed using lysates prepared from BHK cells transfected with the various packaging components. Cultures transfected with $pGRE_5vp16$/BAC/pBSKS(vhs) in the absence of dexamethasone did show VP16 levels intermediate to cultures transfected either with BAC alone (lowest) or those transfected with $pGRE_5vp16$/BAC/pBSKS(vhs) in the presence of 100 nM dexamethasone (highest)(Figure 9C).

VP16-mediated enhancement of packaged amplicon expression titers could be due to increased DNA replication and packaging of amplicon genomes.

Conversely, the additional VP16 that is expressed via $pGRE_5vp16$ could be incorporated into virions and act by increasing vector-directed expression in transduced cells. To test the possibility that VP16 is acting by increasing replication in the packaging cells, concentrations of vector genomes in BAC-derived vector stocks were determined. HSVlac stocks produced in the presence or absence of vhs and/or VP16 were analyzed using a "real-time" quantitative PCR method. The concentration of vector genome was increased two-fold in stocks prepared in the presence of VP16 and this increase was unaffected by the presence of vhs (Figure 10). VP16 expression was induced with 100 nM dexamethasone treatment at varying time points prior to introduction of the packaging components. Dexamethasone-induced production of VP16 prior to transfection of the packaging components did not appear to enhance amplicon titers over that observed with basal $pGRE_5vp16$-mediated expression (Figure 11). This suggests that low levels of VP16 are sufficient to enhance amplicon packaging in the presence of vhs.

Pre-loading of packaging cells with low levels of the potent HSV transcriptional activator VP16 led to a 2- to 5-fold additional increase in amplicon expression titers only in the presence of vhs for cosmid- and BAC-based packaging systems, respectively. This observation indicates the transactivation and structural functions of VP16 were not sufficient to increase viable viral particle production when vhs was absent, and most likely led to generation of incomplete virions containing amplicon genomes as detected by quantitative PCR. When vhs was present for viral assembly, however, VP16-mediated enhancement of genome replication led to higher numbers of viable particles formed. The effect of VP16 on expression titers was not specific to amplicons possessing the immediate-early 4/5 promoter of HSV, as amplicons with other promoters were packaged to similar titers in the presence of VP16 and vhs.

VP16 is a strong transactivator protein and structural component of the HSV virion (Post et al., "Regulation of alpha genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with alpha gene promoters," Cell, **24**:555-565 (1981), which is hereby incorporated by reference). VP16-mediated transcriptional activation occurs via interaction of VP16 and two cellular factors, Oct-1 (O'Hare and Goding, "Herpes simplex virus regulatory elements and the immunoglobulin octamer domain bind a common factor and are both

targets for virion transactivation," Cell, **52**:435-445 (1988); Preston et al., "A complex formed between cell components and an HSV structural polypeptide binds to a viral immediate early gene regulatory DNA sequence," Cell, **52**:425-434 (1988); Stern et al., "The Oct-1 homoeodomain directs formation of a multiprotein-DNA complex with the HSV transactivator VP16," Nature, **341**:624-630 (1989), which are hereby incorporated by reference in their entirety) and HCF (Wilson et al., "The VP 16 accessory protein HCF is a family of polypeptides processed from a large precursor protein," Cell, **74**:115-125 (1993); Xiao and Capone, "A cellular factor binds to the herpes simplex virus type 1 transactivator Vmw65 and is required for Vmw65-dependent protein-DNA complex assembly with Oct-1," Mol. Cell Biol., **10**:4974-4977 (1990), which are hereby incorporated by reference in their entirety), and subsequent binding of the complex to TAATGARAT elements found within HSV IE promoter regions (O'Hare, "The virion transactivator of herpes simplex virus," Semin. Virol., **4**:145-155 (1993), which is hereby incorporated by reference). This interaction results in robust up-regulation of IE gene expression. Neuronal splice-variants of the related Oct-2 transcription factor have been shown to block IE gene activation via binding to TAATGARAT elements (Lillycrop et al., "The octamer-binding protein Oct-2 represses HSV immediate-early genes in cell lines derived from latently infectable sensory neurons," Neuron, **7**:381-390 (1991), which is hereby incorporated by reference), suggesting that cellular transcription factors may also play a role in limiting HSV lytic growth.

The levels of VP16 appear to be important in determining its effect on expression titers. Low, basal levels of VP16 (via uninduced $pGRE_5vp16$) present in the packaging cell prior to introduction of the packaging components induced the largest effect on amplicon expression titers. Conversely, higher expression of VP16 (via dexamethasone-induced $pGRE_5vp16$) did not enhance virus production to the same degree and may have, in fact, abrogated the process. The presence of glucocorticoids in the serum components of growth medium is the most likely reason for this low-level VP16 expression, as charcoal-stripped sera significantly reduces basal expression from this construct. Perhaps only a low level or short burst of VP16 is required to initiate IE gene transcription, but excessive VP16 leads to disruption of the temporal progression through the HSV lytic cycle, possibly via inhibition of vhs activity. Moreover, evidence has arisen to suggest vhs activity is downregulated by

newly synthesized VP16 during the HSV lytic cycle, thereby allowing for accumulation of viral mRNAs after host transcripts have been degraded (Smibert et al., "Herpes simplex virus VP16 forms a complex with the virion host shutoff protein vhs," J. Virol., **68**(4):233-236 (1994); Lam et al., "Herpes simplex virus VP16 rescues viral mRNA from destruction by the virion host shutoff function," EMBO J., **15**:2575-2581 (1996), which are hereby incorporated by reference in their entirety). Therefore, a delicate regulatory protein balance may be required to attain optimal infectious particle propagation. Additionally, the 100-nM dexamethasone treatment used to induce VP16 expression may have a deleterious effect on cellular gene activity and/or interfere with replication of the OriS-containing amplicon genome in packaging cells. High levels of dexamethasone have been shown previously to repress HSV-1 OriS-dependent replication by an unknown mechanism (Hardwicke and Schaffer, "Differential effects of nerve growth factor and dexamethasone on herpes simplex virus type 1 oriL- and oriS-dependent DNA replication in PC12 cells," J. Virol., **71**:3580-3587 (1997), which is hereby incorporated by reference in its entirety).

Example 3 - Examination of Amplicon Cytotoxicity

There is a possibility that addition of viral proteins, like vhs and VP16, to the packaging process may lead to vector stocks that are inherently more cytotoxic. The amplicon stocks described above were examined for cytotoxicity using a lactate dehydrogenase (LDH) release-based cell viability assay. Packaged amplicon stocks were used to transduce NIH 3T3 cells and 48 hours following infection, viability of the cell monolayers was assessed by the LDH-release assay. Amplicon stocks produced in the presence of vhs and VP16 displayed less cytotoxicity on a per virion basis than stocks packaged using the previously published BAC-based protocol (Figure 12) (Stavropoulos and Strathdee, "An enhanced packaging system for helper-dependent herpes simplex virus vectors," J. Virol., **72**:7137-43 (1998), which is hereby incorporated by reference in its entirety)).

Ectopic expression of vhs and VP16 did not lead to amplicon stocks that exhibited higher cytotoxicity than helper virus-free stocks prepared in the traditional manner when examined by an LDH-release assay. Stocks prepared by the

various methods were equilibrated to identical expression titers prior to exposure to cells. The heightened cytotoxicity in stocks produced in the absence of vhs and/or VP16 may reflect that larger volumes of these stocks were required to obtain similar expression titers as the vhs/VP16-containing samples or the levels of defective particles in the former may be significantly higher. Contaminating cellular proteins that co-purify with the amplicon particles are most likely higher in concentration in the traditional stocks, and probably impart the higher toxicity profiles observed.

Example 4 - Comparative Analysis of Helper Virus-Free HSV Amplicon Particles and Helper Virus HSV Amplicon Particles

Helper virus-free HSV amplicon particles were prepared as described above in Example 1 and helper virus-containing HSV amplicon particles were prepared according to known procedures.

Two-dimensional gel analyses were performed on stocks containing the helper virus-free (HVF) virion particles (Figure 14) and helper virus-containing (HVC) virion particles (Figure 15) to determine differences in their protein composition. Virion particles from both helper virus-containing and helper virus-free amplicon stocks were purified by ultracentrifugation on a 30%/60% discontinuous sucrose gradient. Bands containing viral particles were extracted from the gradient at the 30%/60% interface and stored at –80°C until 2-D gel analyses were performed. Prior to gel analyses, protein concentration was determined by the Bradford assay and 100 μg of each sample was resuspended in urea sample buffer (9.5 M ultrapure urea, 2% w/v Nonidet P-40, 5% beta-mercaptoethanol, and 2% ampholines consisting of 1.6% pH 5-7 and 0.4% pH 3.5-10). Fifty μg of each sample was run 2X's on 2-D gels (ampholines pH of 3.5-10), the gels were silver-stained, digitized, and analyzed by comparison of 2-D patterns and spot intensity of helper virus-containing vs. helper virus-free amplicon stocks.

As shown in Table 2 below, the reference spot number, pI, and molecular weight (daltons) are given for polypeptide spots analyzed in the samples obtained from the stocks of HVF and HVC virion particles. Also indicated in Table 2 are the fold increase or decrease (difference) of the polypeptides for gel bands from the two samples. Spot percentages were calculated as individual spot density divided

by total density of all measured spots. The difference is calculated from spot density as follows:

$$\text{Difference} = \frac{(1 - \text{Spot Percentage of HVC})}{(\text{Spot Percentage of HVF})} \times -100$$

A significant increase in the polypeptide spot density is considered to be a difference $\geq +300$, where a significant decrease in the polypeptide spot density is considered to be a difference ≤ -67. Significantly increased and decreased polypeptide spots are highlighted (outlined) in Figures 16A-B and 17A-C, respectively.

Table 2 : Summary of Two-Dimensional Gel Protein Analysis

	Helper Virus-Free			Helper Virus-Containing	
Spot No.	pI	MW	Spot Percent	Spot Percent	Difference
1	6.04	150,730	0.24	0.05	-79
2	6.14	121,290	0.02	0.09	341
3	5.94	103,956	0.61	0.01	-99
4	5.74	96,220	0.34	0.17	-49
5	6.02	93,124	0.07	0.03	-55
6	5.1	92,212	0.71	0.36	-49
7	5.59	89,821	0.00	0.18	66661
8	5.6	87,909	0.02	0.06	220
9	6.28	87,423	0.44	0.03	-93
10	5.48	85,649	0.00	0.05	3970
11	5.92	83,910	0.96	0.14	-85
12	6.97	83,902	0.01	0.15	1032
13	6.59	83,729	0.18	0.01	-97
14	6.7	83,729	0.02	0.61	3080
15	5.53	79,043	5.94	0.99	-83
16	6.06	77,562	1.91	0.48	-75
17	5.68	77,304	0.06	0.00	-100
18	5.76	76,957	0.19	0.00	-99
19	6.31	76,697	0.02	0.02	-8
20	5.98	90,963	0.63	3.27	421
21	6.4	74,967	0.78	7.29	840
22	7.19	74,742	0.10	0.05	-53
23	5.89	72,089	0.09	0.01	-88
24	5.87	70,698	0.02	0.00	-94
25	5.7	70,177	0.19	0.01	-94
26	7.08	70,482	0.03	0.09	235
27	5.36	68,090	0.04	0.06	57
28	6.21	68,220	0.09	0.00	-99
29	6.29	67,874	0.05	0.03	-38
30	6.67	67,406	0.01	0.25	2639

Table 2 cont.

	Helper Virus-Free			Helper Virus-Containing	
Spot No.	pI	MW	Spot Percent	Spot Percent	Difference
31	5.75	66,526	0.03	0.01	-76
32	7.31	68,097	0.12	0.40	239
33	5.52	65,483	0.12	3.41	2693
34	6.08	65,279	2.04	0.19	-91
35	4.99	64,885	0.45	0.41	-9
36	7.39	66,052	0.02	0.11	375
37	7.48	64,007	0.00	0.32	14050
38	6.17	62,165	0.01	0.22	3946
39	6.22	61,473	0.02	0.12	676
40	5.43	61,136	5.90	1.38	-77
41	5.96	61,136	3.24	2.28	-30
42	6.3	61,127	0.27	0.46	69
43	6.42	61,784	0.16	0.11	-31
44	6.74	62,286	0.06	0.06	-8
45	8.44	61,726	0.02	0.79	4651
46	5.61	59,227	0.02	0.02	-12
47	6.48	58,874	0.52	0.22	-57
48	6.59	58,365	3.00	2.01	-33
49	5.28	57,586	0.00	0.04	++++
50	5.71	57,586	0.13	0.02	-89
51	5.57	56,355	0.08	0.02	-73
52	7.48	57,859	0.07	0.02	-68
53	5.02	55,634	0.04	0.20	366
54	8.08	57,487	0.00	0.52	++++
55	6.76	55,915	0.00	0.06	33872
56	7.63	57,152	0.08	0.15	81
57	6.83	55,786	0.00	0.12	9161
58	6.9	55,658	0.05	1.59	3038
59	5.48	54,714	0.17	0.11	-38
60	7.1	56,317	0.01	0.10	1799
61	7.48	56,189	0.01	0.03	412
62	8.28	56,540	0.02	0.30	1849
63	5.01	53,293	0.01	0.14	2347
64	6.29	53,761	0.07	0.04	-42
65	7.09	54,647	0.06	0.05	-28
66	8.54	54,366	1.44	0.39	-73
67	6.12	53,106	0.22	0.01	-98
68	6.68	53,208	0.10	0.11	11
69	6.26	52,582	0.11	0.01	-92
70	5.57	51,842	2.29	0.48	-79
71	6.06	51,926	0.07	0.00	-100
72	5.71	51,295	0.60	0.12	-80
73	6.58	51,403	0.25	0.11	-58
74	6.12	50,615	0.02	0.04	160
75	5.05	49,049	0.31	0.02	-94
76	5.64	49,790	0.07	0.07	8
77	7.06	51,693	0.05	0.00	-92
78	4.97	48,610	0.13	0.06	-57
79	5.59	49,380	0.06	0.09	44

Table 2 cont.

	Helper Virus-Free			Helper Virus-Containing	
Spot No.	pI	MW	Spot Percent	Spot Percent	Difference
80	8.68	50,067	0.05	0.01	-82
81	5.35	47,876	0.09	0.01	-88
82	5.6	47,055	0.21	0.05	-75
83	5.16	45,244	0.23	0.06	-74
84	8.79	47,487	0.15	0.40	167
85	8.66	47,344	0.06	0.08	34
86	5.67	45,961	0.23	0.05	-81
87	6.67	47,149	0.00	0.85	33868
88	6.59	47,020	0.01	0.41	6309
89	6.26	46,289	0.21	0.02	-90
90	5.79	45,277	0.54	0.05	-91
91	6.47	46,027	0.09	0.14	51
92	5.3	44,867	0.18	0.04	-77
93	8.15	46,934	0.13	0.10	-26
94	7.39	46,426	0.00	0.07	10326
95	5.99	44,836	0.01	0.10	2005
96	7.11	45,912	0.22	0.46	109
97	5.31	42,479	0.29	0.06	-80
98	7.48	44,885	0.01	0.11	1789
99	8.59	46,413	0.65	3.08	377
100	8.74	46,413	0.81	0.28	-65
101	5.69	42,870	0.15	0.49	227
102	8.46	44,092	0.21	1.50	617
103	5.91	42,296	1.30	2.59	99
104	6.14	42,491	0.05	0.07	63
105	5.33	41,888	1.11	0.81	-27
106	7.39	45,972	0.02	0.08	409
107	6.29	42,187	0.11	0.02	-81
108	7.97	42,453	1.24	0.92	-26
109	6.19	41,629	0.05	0.00	-100
110	7.74	42,193	0.16	0.49	211
111	7.46	41,779	0.16	0.01	-94
112	6.28	41,122	0.03	0.31	1004
113	7.57	41,828	0.13	0.23	80
114	6.13	40,666	0.21	0.02	-92
115	8.78	40,105	0.11	0.51	364
116	7.57	40,735	0.03	0.00	-96
117	5.39	39,543	0.10	0.01	-96
118	6.56	40,020	0.04	0.02	-61
119	5.33	39,135	0.05	0.00	-100
120	7.49	40,094	0.17	0.13	-24
121	6.81	39,557	0.36	0.14	-60
122	7.64	39,903	0.05	0.28	439
123	6.42	38,992	0.15	0.00	-100
124	6.38	38,536	0.13	0.10	-23
125	7.42	38,728	0.03	0.16	528
126	7.17	38,056	0.09	0.14	61
127	5.6	36,841	0.01	0.07	1279
128	5.13	35,384	0.00	0.11	++++
129	5.98	36,178	0.00	0.43	45454

Table 2 cont.

	Helper Virus-Free			Helper Virus-Containing	
Spot No.	pI	MW	Spot Percent	Spot Percent	Difference
130	7.52	37,007	0.21	0.00	-100
131	5.42	35,924	0.17	0.03	-85
132	7.71	36,520	0.02	0.33	2141
133	5.62	35,516	0.03	0.15	473
134	7.18	36,349	0.09	0.23	153
135	4.99	34,526	0.33	0.05	-84
136	5.98	35,312	0.19	0.09	-50
137	6.39	35,645	0.03	0.66	1837
138	6.05	35,544	0.67	0.21	-69
139	5.73	35,006	0.03	0.01	-76
140	5.02	33,830	0.53	0.21	-60
141	8.04	35,162	3.36	7.90	135
142	7.55	35,584	0.05	0.35	553
143	6.57	34,883	0.04	0.47	1204
144	6	34,316	0.12	0.01	-92
145	6.06	34,479	0.03	0.14	396
146	5.51	33,986	1.43	6.43	349
147	5.14	32,919	0.55	1.79	225
148	6.23	34,225	0.32	0.18	-45
149	6.65	34,318	0.00	0.26	14364
150	6.54	33,855	0.06	0.08	40
151	8.64	31,837	0.36	0.07	-79
152	6.07	32,856	0.24	0.48	96
153	6.27	32,856	0.01	0.18	1132
154	8.83	31,493	0.39	0.13	-68
155	5.14	31,043	0.00	0.14	++++
156	5.29	31,794	0.01	0.16	2152
157	7.37	32,005	0.03	0.01	-72
158	6.69	31,595	0.11	0.00	-100
159	6.08	31,233	0.04	0.33	697
160	6.56	31,287	0.02	0.12	409
161	4.99	30,334	0.45	0.00	-99
162	5.72	30,214	0.01	0.17	3364
163	5.18	30,157	0.00	0.20	6047
164	6.52	30,619	0.00	0.03	23471
165	5.63	30,329	0.05	0.38	686
166	6.46	29,610	0.43	0.15	-66
167	6.75	29,643	0.33	0.17	-49
168	6.28	29,186	0.22	0.85	285
169	8.48	30,519	0.98	0.00	-100
170	6.07	28,978	1.99	0.43	-78
171	5.33	29,767	0.08	0.15	87
172	7.88	28,993	0.33	0.09	-73
173	6.6	28,890	0.00	0.10	6034
174	7.45	28,896	0.24	0.42	72
175	6.86	28,657	0.00	0.18	197412
176	7.23	28,654	0.00	0.39	145023
177	6.98	28,210	0.05	0.01	-74
178	6.47	27,932	0.03	0.15	452
179	6.64	27,992	0.26	0.88	247

Table 2 cont.

	Helper Virus-Free			Helper Virus-Containing	
Spot No.	pI	MW	Spot Percent	Spot Percent	Difference
180	6.24	27,822	0.05	0.02	-72
181	6.11	27,639	0.55	0.00	-100
182	6.39	27,639	0.01	0.15	2823
183	6.74	27,677	0.05	0.06	16
184	7.17	27,827	0.22	0.87	295
185	6.45	27,347	0.02	0.55	2959
186	7.65	27,379	0.12	0.04	-68
187	6.29	26,871	0.22	0.09	-59
188	6.17	26,834	0.84	0.13	-84
189	5.36	26,421	0.37	0.02	-95
190	6.61	26,767	0.34	0.21	-38
191	5	25,206	2.23	0.18	-92
192	5.69	26,122	0.08	0.90	978
193	5.95	26,047	0.55	2.48	350
194	6.67	26,347	0.34	0.00	-100
195	6.57	26,312	0.13	0.00	-99
196	5.33	25,186	0.00	0.09	2843
197	5.13	24,166	0.06	0.00	-97
198	6.56	25,542	0.20	0.00	-99
199	5.91	24,812	0.35	0.04	-88
200	6.2	24,931	0.32	0.03	-90
201	6.72	25,122	0.32	0.36	13
202	5.45	24,363	0.08	0.03	-63
203	5.29	24,326	0.14	0.21	53
204	8.69	23,726	0.16	0.04	-78
205	9.31	22,854	0.05	0.04	-28
206	7.81	24,487	0.30	0.49	67
207	6.58	24,212	0.33	0.12	-65
208	6.07	23,906	0.22	0.00	-100
209	9.06	22,562	0.12	0.04	-64
210	7.55	24,313	0.08	0.04	-49
211	6.36	23,723	5.41	0.97	-82
212	8.45	23,160	0.10	0.03	-71
213	7.68	23,407	0.07	0.02	-76
214	6.71	23,127	0.01	0.72	5995
215	6.09	22,699	0.29	0.00	-100
216	5.01	20,971	0.27	0.25	-5
217	6.66	22,567	0.10	0.08	-20
218	5.42	21,406	2.46	1.17	-52
219	6.43	21,381	0.20	0.17	-16
220	4.61	19,596	2.30	1.27	-45
221	6.62	21,063	0.19	0.08	-56
222	7.7	21,143	0.67	0.20	-71
223	8.81	19,769	0.23	0.05	-81
224	6.18	20,173	1.76	0.06	-97
225	7.19	20,828	0.72	0.06	-92
226	6.78	20,503	0.01	0.10	679
227	6.98	20,433	1.18	0.57	-52
228	5.28	19,348	0.14	0.03	-81
229	5.31	18,787	0.10	0.05	-49

Table 2 cont.

	Helper Virus-Free			Helper Virus-Containing	
Spot No.	pI	MW	Spot Percent	Spot Percent	Difference
230	5.93	18,712	0.76	0.00	-100
231	5.64	18,600	0.31	0.22	-28
232	6.67	19,523	0.14	0.11	-20
233	8.59	18,575	1.65	5.90	259
234	5.07	17,292	2.11	0.73	-66
235	6	18,046	0.00	0.01	6403
236	8.95	18,029	0.11	0.05	-58
237	5.4	17,776	0.49	0.00	-99
238	5.21	17,627	0.01	0.15	1079
239	4.96	16,512	1.00	0.17	-83
240	8.79	17,586	0.10	0.65	562
241	6.55	17,843	0.05	0.01	-87
242	6.69	17,703	0.03	0.11	222
243	6.83	17,213	0.10	0.15	59
244	8.68	16,051	1.61	0.01	-99
245	7.4	16,897	0.02	0.21	824
246	6.25	15,855	0.27	0.10	-64
247	6.23	15,342	0.25	0.71	180
248	7.25	16,345	0.05	0.06	12
249	6.04	15,269	0.01	0.21	2260
250	7.11	15,932	0.07	0.03	-61
251	nd	nd	0.26	1.52	496
252	6.69	14,760	0.22	0.51	136
253	7.32	14,729	2.34	0.82	-65
254	nd	nd	0.07	0.46	598
255	nd	nd	1.39	0.03	-98

nd = not determined; ++++ = greater than 200,000

Based on the number of differences in the 2D gels for HVF and HVC virion particle polypeptide analyses and the different size and morphology of the HVF virion particles shown in Figure 13 (as compared to particles produced using helper virus), it is clear the HSV amplicon particles produced according to the present invention are different in kind from the HSV amplicon particles produced using a helper virus in accordance with previously known techniques.

Although the invention has been described in detail for purposes of illustration, it is to be understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED IS:

1. A method for producing herpes simplex virus (HSV) amplicon particles, comprising:

co-transfecting a host cell with the following:

(i) an amplicon vector comprising an HSV origin of replication, an HSV cleavage/packaging signal, and a heterologous transgene expressible in a patient,

(ii) one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals, and

(iii) a vhs expression vector encoding a virion host shutoff protein; and

isolating HSV amplicon particles produced by the host cell, the HSV amplicon particles including the transgene.

2. The method according to claim 1, wherein the isolated HSV amplicon particles are substantially pure.

3. The method according to claim 1, wherein the virion host shutoff protein is selected from the group consisting of HSV-1 virion host shutoff protein, HSV-2 virion host shutoff protein, HSV-3 virion host shutoff protein, bovine herpesvirus 1 virion host shutoff protein, bovine herpesvirus 1.1 virion host shutoff protein, gallid herpesvirus 1 virion host shutoff protein, gallid herpesvirus 2 virion host shutoff protein, suid herpesvirus 1 virion host shutoff protein, baboon herpesvirus 2 virion host shutoff protein, pseudorabies virus virion host shutoff protein, cercopithecine herpesvirus 7 virion host shutoff protein, meleagrid herpesvirus 1 virion host shutoff protein, equine herpesvirus 1 virion host shutoff protein, and equine herpesvirus 4 virion host shutoff protein.

4. The method according to claim 3, wherein the virion host shutoff protein is selected from the group consisting of HSV-1 virion host shutoff protein, HSV-2 virion host shutoff protein, and HSV-3 virion host shutoff protein.

5. The method according to claim 4, wherein the vhs expression vector comprises:

a DNA molecule encoding the HSV virion host shutoff protein operatively coupled to its native transcriptional control elements.

6. The method according to claim 1, wherein the vhs expression vector comprises:

a DNA molecule encoding the virion host shutoff protein;

a promoter element operatively coupled 5' to the DNA molecule; and

a transcription termination element operatively coupled 3' to the DNA molecule.

7. The method according to claim 1, wherein the host cell expresses a VP16 protein.

8. The method according to claim 7, wherein the VP16 protein is selected from the group consisting of HSV-1 VP16, HSV-2 VP16, bovine herpesvirus 1 VP16, bovine herpesvirus 1.1 VP16, gallid herpesvirus 1 VP16, gallid herpesvirus 2 VP16, meleagrid herpesvirus 1 VP16, and equine herpesvirus 4 VP16.

10. The method according to claim 7 further comprising:

transfecting the host cell, prior to said co-transfecting, with a vector encoding the VP16 protein.

11. The method according to claim 10, wherein said transfecting is carried out at least about 4 hours prior to said co-transfecting.

12. The method according to claim 7, wherein the host cell stably expresses the VP16 protein.

13. The method according to claim 1, wherein the isolated HSV amplicon particles are present at a concentration of greater than 1 x 10^6 particles per milliliter.

14. The method according to claim 1 further comprising:
concentrating the isolated HSV amplicon particles to a concentration of at least about 1 x 10^7 particles per milliliter.

15. The method according to claim 1 wherein the transgene encodes a therapeutic transgene product.

16. The method according to claim 15, wherein the therapeutic transgene product is a protein or an RNA molecule.

17. The method according to claim 16, wherein the therapeutic transgene product is an RNA molecule selected from the group consisting of antisense RNA, RNAi, and an RNA ribozyme.

18. The method according to claim 16, wherein the therapeutic transgene product is a protein selected from the group consisting of receptors, signaling molecules, transcription factors, growth factors, apoptosis inhibitors, apoptosis promoters, DNA replication factors, enzymes, structural proteins, neural proteins, and histone or non-histone proteins.

19. An HSV amplicon particle produced according to the process of claim 1.

20. An HSV amplicon particle produced according to the process of claim 15.

21. A system for preparing HSV amplicon particles comprising:

an amplicon vector comprising an HSV origin of replication, an HSV cleavage/packaging signal, and a transgene insertion site;

one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals; and

a vhs expression vector encoding a virion host shutoff protein;

wherein upon introduction of the system into a host cell, the host cell produces herpes simplex virus amplicon particles.

22. The system according to claim 21 further comprising:

the host cell, which stably expresses a VP16 protein.

23. The system according to claim 22, wherein the VP16 protein is selected from the group consisting of HSV-1 VP16, HSV-2 VP16, bovine herpesvirus 1 VP16, bovine herpesvirus 1.1 VP16, gallid herpesvirus 1 VP16, gallid herpesvirus 2 VP16, meleagrid herpesvirus 1 VP16, and equine herpesvirus 4 VP16.

24. The system according to claim 21 further comprising:

a vector encoding the VP16 protein.

25. The system according to claim 24, wherein the VP16 protein is selected from the group consisting of HSV-1 VP16, HSV-2 VP16, bovine herpesvirus 1 VP16, bovine herpesvirus 1.1 VP16, gallid herpesvirus 1 VP16, gallid herpesvirus 2 VP16, meleagrid herpesvirus 1 VP16, and equine herpesvirus 4 VP16.

26. The system according to claim 21, wherein the virion host shutoff protein is selected from the group consisting of HSV-1 virion host shutoff protein, HSV-2 virion host shutoff protein, HSV-3 virion host shutoff protein, bovine herpesvirus 1 virion host shutoff protein, bovine herpesvirus 1.1 virion host shutoff protein, gallid herpesvirus 1 virion host shutoff protein, gallid herpesvirus 2 virion host shutoff protein, suid herpesvirus 1 virion host shutoff protein, baboon herpesvirus 2 virion host shutoff protein, pseudorabies virus virion host shutoff protein, cercopithecine herpesvirus 7 virion host shutoff protein, meleagrid

herpesvirus 1 virion host shutoff protein, equine herpesvirus 1 virion host shutoff protein, and equine herpesvirus 4 virion host shutoff protein.

27. The system according to claim 26, wherein the virion host shutoff protein is selected from the group consisting of HSV-1 virion host shutoff protein, HSV-2 virion host shutoff protein, and HSV-3 virion host shutoff protein.

28. The system according to claim 27, wherein the vhs expression vector comprises:

a DNA molecule encoding a HSV vhs protein operatively coupled to its native transcriptional control elements.

29. The system according to claim 21, wherein the vhs expression vector comprises:

a DNA molecule encoding the virion host shutoff protein;

a promoter element operatively coupled 5' to the DNA molecule; and

a transcription termination element operatively coupled 3' to the DNA molecule.

30. A kit for preparing HSV amplicon particles comprising:

an amplicon vector comprising an HSV origin of replication, an HSV cleavage/packaging signal, and a transgene insertion site;

one or more vectors individually or collectively encoding all essential HSV genes but excluding all cleavage/packaging signals;

a vhs expression vector encoding an virion host shutoff protein;

a population of host cells susceptible to transfection by the amplicon vector, the vhs expression vector, and the one or more vectors; and

directions for transfecting the host cells under conditions to produce HSV amplicon particles.

31. The kit according to claim 30 further comprising:

a vector encoding a VP16 protein.

32. The kit according to claim 31, wherein the VP16 protein is selected from the group consisting of HSV-1 VP16, HSV-2 VP16, bovine herpesvirus 1 VP16, bovine herpesvirus 1.1 VP16, gallid herpesvirus 1 VP16, gallid herpesvirus 2 VP16, meleagrid herpesvirus 1 VP16, and equine herpesvirus 4 VP16.

33. The kit according to claim 30, wherein the host cell stably expresses a VP16 protein.

34. The kit according to claim 33, wherein the VP16 protein is selected from the group consisting of HSV-1 VP16, HSV-2 VP16, bovine herpesvirus 1 VP16, bovine herpesvirus 1.1 VP16, gallid herpesvirus 1 VP16, gallid herpesvirus 2 VP16, meleagrid herpesvirus 1 VP16, and equine herpesvirus 4 VP16.

35. The kit according to claim 30, wherein the virion host shutoff protein is selected from the group consisting of HSV-1 virion host shutoff protein, HSV-2 virion host shutoff protein, HSV-3 virion host shutoff protein, bovine herpesvirus 1 virion host shutoff protein, bovine herpesvirus 1.1 virion host shutoff protein, gallid herpesvirus 1 virion host shutoff protein, gallid herpesvirus 2 virion host shutoff protein, suid herpesvirus 1 virion host shutoff protein, baboon herpesvirus 2 virion host shutoff protein, pseudorabies virus virion host shutoff protein, cercopithecine herpesvirus 7 virion host shutoff protein, meleagrid herpesvirus 1 virion host shutoff protein, equine herpesvirus 1 virion host shutoff protein, and equine herpesvirus 4 virion host shutoff protein.

36. The kit according to claim 35, wherein the virion host shutoff protein is selected from the group consisting of HSV-1 virion host shutoff protein, HSV-2 virion host shutoff protein, and HSV-3 virion host shutoff protein.

37. The kit according to claim 36, wherein the vhs expression vector comprises:

a DNA molecule encoding the HSV virion host shutoff protein operatively coupled to its native transcriptional control elements.

38. The kit according to claim 30, wherein the vhs expression vector comprises:

a DNA molecule encoding the virion host shutoff protein;

a promoter element operatively coupled 5' to the DNA molecule; and

a transcription termination element operatively coupled 3' to the DNA molecule.

39. A method of treating a neurological disease or disorder comprising:

providing HSV amplicon particles according to claim 20 and

exposing neural or pre-neural cells of a patient to the HSV amplicon particles under conditions effective for infective transformation of the neural or pre-neural cells, wherein the therapeutic transgene product is expressed *in vivo* in the neural or pre-neural cells, thereby treating the neurological disease or disorder.

40. The method according to claim 39, wherein said exposing is carried out *ex vivo* using pre-neural cells, said method further comprising:

introducing transformed pre-neural cells into the patient.

41. The method according to claim 39, wherein said exposing is carried out *in vivo* by administering the HSV amplicon particles directly to neural cells.

42. The method according to claim 41, wherein said administering comprises intraparenchymal, intramuscular, intravenous, intracerebroventricular, subcutaneous, or intramucosal delivery.

43. The method according to claim 39, wherein the neurological disease or disorder is a lysosomal storage disease, Lesch-Nyhan syndrome, amyloid polyneuropathy, Alzheimer's Disease, retinoblastoma, Duchenne's muscular dystrophy, Parkinson's Disease, Diffuse Lewy Body disease, stroke, brain tumor, epilepsy, or arteriovascular malformation.

44. The method according to claim 39, wherein the therapeutic transgene product is a protein or an RNA molecule.

45. The method according to claim 44, wherein the therapeutic transgene product is an RNA molecule selected from the group consisting of antisense RNA, RNAi, and an RNA ribozyme.

46. The method according to claim 44, wherein the therapeutic transgene product is a protein selected from the group consisting of receptors, signaling molecules, transcription factors, growth factors, apoptosis inhibitors, apoptosis promoters, DNA replication factors, enzymes, structural proteins, neural proteins, and histone or non-histone proteins.

47. The method according to claim 39, wherein the HSV amplicon particles are present in a pharmaceutically acceptable carrier.

48. The method according to claim 39, wherein the patient is a mammal.

49. The method according to claim 39, wherein the patient is a human.

50. A method of inhibiting development of a neurological disease or disorder comprising:

providing HSV amplicon particles according to claim 20 and

exposing neural cells of a patient susceptible to development of a neurological disease or disorder to the HSV amplicon particles under conditions effective for infective transformation of the neural cells of the patient, wherein the therapeutic transgene product is expressed *in vivo* in the neural cells, thereby inhibiting development of the neurological disease or disorder.

51. The method according to claim 50, wherein said exposing is carried out *ex vivo* using neural stem cells, said method further comprising:

introducing transformed neural stem cells into the patient.

52. The method according to claim 50, wherein said exposing is carried out *in vivo* by administering the HSV amplicon particles directly to the neural cells.

53. The method according to claim 52, wherein said administering comprises intraparenchymal, intramuscular, intravenous, intracerebroventricular, subcutaneous, or intramucosal delivery.

54. The method according to claim 50, wherein the neurological disease or disorder is a lysosomal storage disease, Lesch-Nyhan syndrome, amyloid polyneuropathy, Alzheimer's Disease, retinoblastoma, Duchenne's muscular dystrophy, Parkinson's Disease, Diffuse Lewy Body disease, stroke, brain tumor, epilepsy, or arteriovascular malformation.

55. The method according to claim 50, wherein the therapeutic transgene product is a protein or an RNA molecule.

56. The method according to claim 55, wherein the therapeutic transgene product is an RNA molecule selected from the group consisting of antisense RNA, RNAi, and an RNA ribozyme.

57. The method according to claim 55, wherein the therapeutic transgene product is a protein selected from the group consisting of receptors, signaling molecules, transcription factors, growth factors, apoptosis inhibitors, apoptosis promoters, DNA replication factors, enzymes, structural proteins, neural proteins, and histone or non-histone proteins.

58. The method according to claim 50, wherein the HSV amplicon particles are present in a pharmaceutically acceptable carrier.

59. The method according to claim 50, wherein the patient is a mammal.

60. The method according to claim 50, wherein the patient is a human.

61. A method of expressing a therapeutic gene product in a patient comprising:

providing HSV amplicon particles according to claim 20 and exposing patient cells to the HSV amplicon particles under conditions effective for infective transformation of the cells, wherein the therapeutic transgene product is expressed *in vivo* in transformed cells.

62. The method according to claim 61, wherein said exposing is carried out *ex vivo*, said method further comprising:

introducing transformed cells into the patient.

63. The method according to claim 61, wherein said exposing is carried out *in vivo* by administering the HSV amplicon particles directly to the patient cells which are to be transformed.

64. The method according to claim 63, wherein said administering comprises intraparenchymal, intramuscular, intravenous, intracerebroventricular, subcutaneous, or intramucosal delivery.

65. The method according to claim 61, wherein the therapeutic transgene product is a protein or an RNA molecule.

66. The method according to claim 65, wherein the therapeutic transgene product is an RNA molecule selected from the group consisting of antisense RNA, RNAi, and an RNA ribozyme.

67. The method according to claim 65, wherein the therapeutic transgene product is a protein selected from the group consisting of receptors, signaling molecules, transcription factors, growth factors, apoptosis inhibitors, apoptosis promoters, DNA replication factors, enzymes, structural proteins, neural proteins, and histone or non-histone proteins.

68. The method according to claim 61, wherein the HSV amplicon particles are present in a pharmaceutically acceptable carrier.

69. The method according to claim 61, wherein the patient is a mammal.

70. The method according to claim 61, wherein the patient is a human.

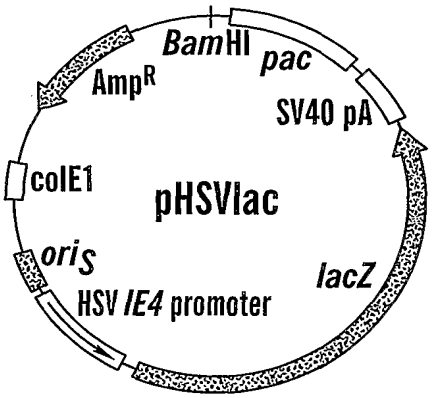

BamHI
pac
Amp^R
SV40 pA
colE1
pHSVlac
ori_S
lacZ
HSV IE4 promoter

FIG. 1A

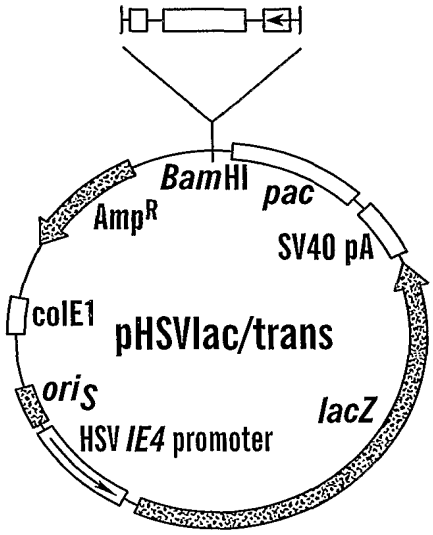

BamHI
pac
Amp^R
SV40 pA
colE1
pHSVlac/trans
ori_S
lacZ
HSV IE4 promoter

FIG. 1B

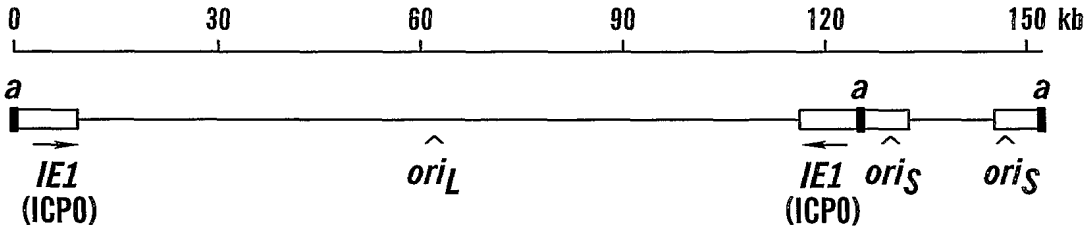


FIG. 2A

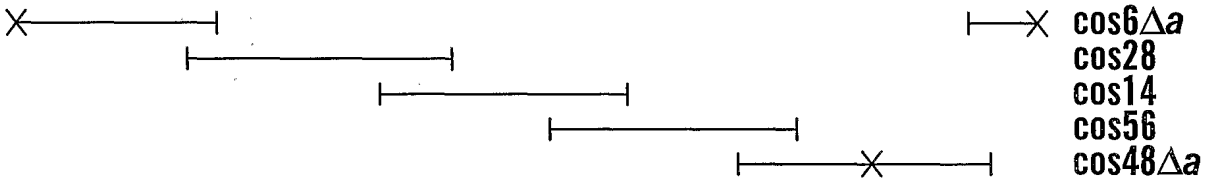


FIG. 2B

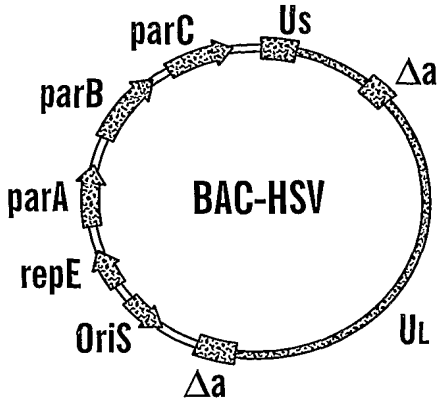


FIG. 3

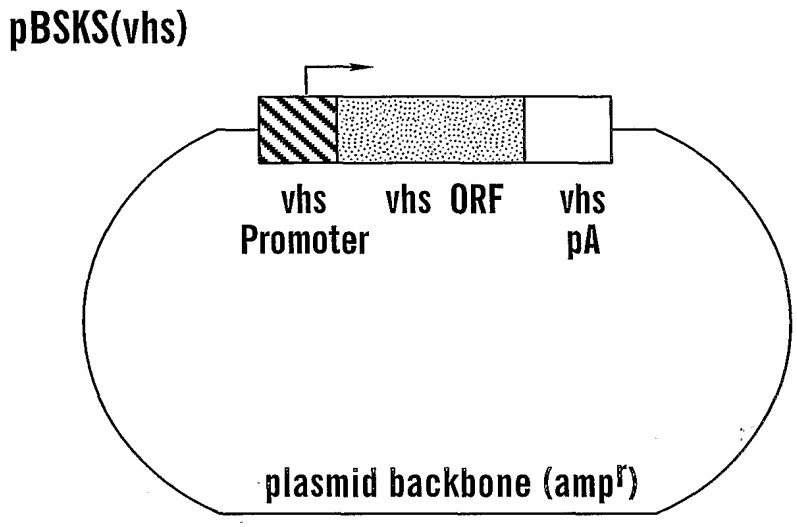

pBSKS(vhs)
vhs Promoter
vhs ORF
vhs pA
plasmid backbone (amp^r)

FIG. 4A

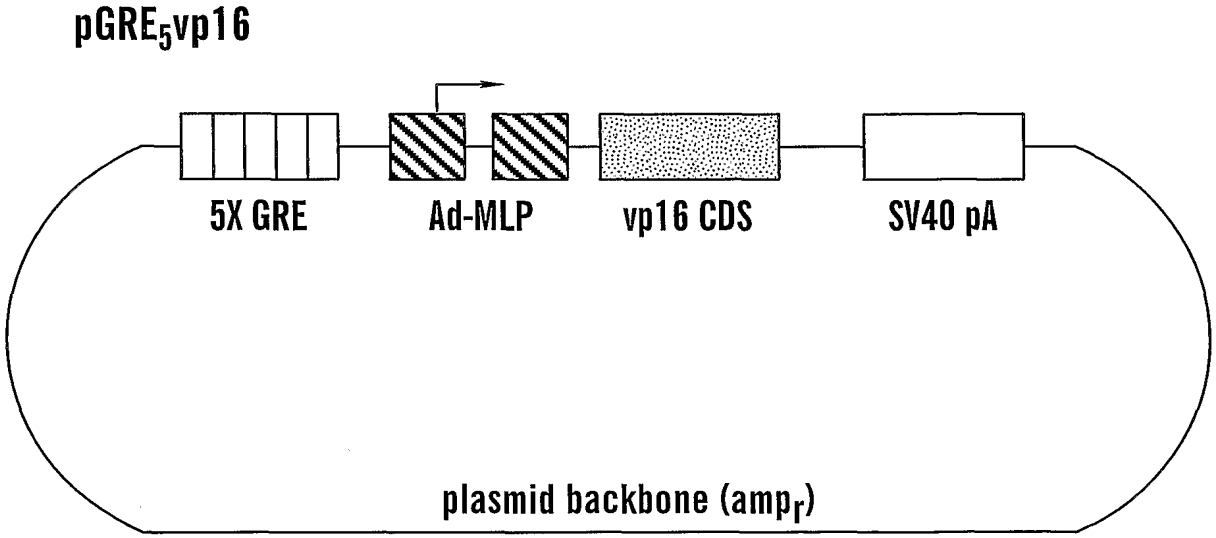

pGRE$_5$vp16
5X GRE
Ad-MLP
vp16 CDS
SV40 pA
plasmid backbone (amp$_r$)

FIG. 5

CGTCGACGACCACAGAGAAGGTGCGATGGGTATTTTCCCCGTACACCGTCTTGGCGTTGG
CGGCCGCCTGGCCCGCCTTGGTGAGCGCGTTGGACAGGATCTGGACCTGGGTGCTGGTGC
TGGACGACACGCCCTCCTCGCGGGCAGCAAAGGTGACGCAGGTACTCGTGGTGAACACGG
AAAATTTGCCGTTAACCCCGAGCTCGAACGTGGTGGGCGTGGCACTATCGGCCCCGGTCG
CGTTAAGGACCTTGGTGAGCTGCGGCCTCGTCAGGCGCAACTGAACGTCGGGGGTTCCCT 300
GGGGAACCAGCACCACAAAGCTCGTCAGTTCGCGCTTCATCAGCGTCTCGCTGGCTAGCT
CAACGGCCTCGCCGTCGGACGTCGTCGTCCATATGCGCTGAACCAGCGTGCGAAACGGGG
CCTGGCCCGTGATCGCCAACTCCACCCGACGTAGGTCCGGGTACTGGTTGGCGCGAAACA
CGCTCAGGAGGGAGCGCTTCTGGTCCACGAGAGACAGGAACGCCGCCGTGGGTCCGCGCC
AGCGATACCGACTGAATTGCGAGTGTTCCAGGGGCAGGAACACCTGCTCCCCAAAGATCG 600
TGTTATGGATAAGGATGCCCCGGTCGCCCATAACCAGAAGCGAGTCCAGAAGGCTCGTGC
GCAGCGGGGCAAACGCCTGTAGGATTCCATTAAGTTCGGCGCCCTGCAGGACCACCTGGC
AGGGCGCCCCCTCCTCCGGCTGCCCGAGGGACGCGTCCGACGCGTCCTCCACGGGGGAGG
CGGGGGCCACACCGCCAGGGGAATCCGTCATCCCAACGCGGGCTGGGAACACCCCACAGT
GACGAGGTGGGCTTCGGTGGTGAGGGCAGCCGGGCCGGGGTCTCGGGTGCGGGACGCGGA 900
GGGGGCGTATGCCGCTGCGAGGGTGGGGTTTTGATGGCAGCCAGGGGACCCAAGCAACCG
GACCGTCGCTCACCGAGCCAGAAACTACGGCAGGCCCGCCGCGCTAGCCTGATTAAATAC
GCCCCCAGCTCGTTAGGCCACACCCTTTTGGAAGAGGCAATGAGCGGGGGGAAGGTTGGC
CCGCACCGGCGCATGCAGGGTGCTGCACCAATCCGCGTGGAGTTGGGCCATCGAAATTAT
AAAGAGCGTCCCCTAACGGATTATTGTCCTCTTGTGTCGGTGTTGTTGTCTGGGTCACCA 1200
TACACAGAGAGACAGGCTCGGGTGTCCCGGACCGTCGCACCAACCACGCCTTAGTTAGGC
CGATCCGCAGTTACAATTGACCTGACATGGGTTTGTTCGGGATGATGAAGTTTGCCCACA
CACACCATCTGGTCAAGCGCCGGGGCCTTGGGGCCCCGGCCGGGTACTTCACCCCCATTG
CCGTGGACCTGTGGAACGTCATGTACACGTTGGTGGTCAAATATCAGCGCCGATACCCCA
GTTACGACCGCGAGGCCATTACGCTACACTGCCTCTGTCGCTTATTAAAGGTGTTTACCC 1500
AAAAGTCCCTTTTCCCCATCTTCGTTACCGATCGCGGGGTCAATTGTATGGAGCCGGTTG
TGTTTGGAGCCAAGGCCATCCTGGCCCGCACGACGGCCCAGTGCCGGACGGACGAGGAGG
CCAGTGACGTGGACGCCTCTCCACCGCCTTCCCCCATCACCGACTCCAGACCCAGCTCTG
CCTTTTCCAACATGCGCCGGCGCGGCACCTCTCTGGCCTCGGGGACCCGGGGGACGGCCG
GGTCCGGAGCCGCGCTGCCGTCCGCCGCGCCCTCGAAGCCGGCCCTGCGTCTGGCGCATC 1800
TGTTCTGTATTCGCGTTCTCCGGGCCCTGGGGTACGCCTACATTAACTCGGGTCAGCTGG
AGGCGGACGATGCCTGCGCCAACCTCTATCACACCAACACGGTCGCGTACGTGTACACCA
CGGACACTGACCTCCTGTTGATGGGCTGTGATATTGTGTTGGATATTAGCGCCTGCTACA
TTCCCACGATCAACTGTCGCGATATACTAAAGTACTTTAAGATGAGCTACCCCCAGTTCC
TGGCCCTCTTTGTCCGCTGCCACACCGACCTCCATCCCAATAACACCTACGCCTCCGTGG 2100
AGGATGTGCTGCGCGAATGTCACTGGACCCCCCCGAGTCGCTCTCAGACCCGGCGGGCCA
TCCGCCGGGAACACACCAGCTCGCGCTCCACGGAAACCAGGCCCCCTCTGCCGCCGGCCG
CCGGCGGCACCGAGACGCGCGTCTCGTGGACCGAAATTCTAACCCAACAGATCGCCGGCG
GATACGAAGACGACGAGGACCTCCCCCTGGATCCCCGGGACGTTACCGGGGGCCACCCCG
GCCCCAGGTCGTCCTCCTCGGAGATACTCACCCCGCCCGAGCTCGTCCAGGTCCCGAACG 2400
CGCAGCTGCTGGAAGAGCACCGCAGTTATGTGGCCAACCCGCGACGCCACGTCATCCACG
ACGCCCCAGAGTCCCTGGACTGGCTCCCCGATCCCATGACCATCACCGAGCTGGTGGAAC
ACCGCTACATTAAGTACGTCATATCGCTTATCGGCCCCAAGGAGCGGGGGCCGTGGACTC
TTCTGAAACGCCTGCCTATCTACCAGGACATCCGCGACGAAAACCTGGCGCGATCTATCG
TGACCCGGCATATCACGGCCCCTGATATCGCCGACAGGTTTCTGGAGCAGTTGCGGACCC 2700

FIG. 4B

AGGCCCCCCCCACCCGCGTTCTACAAGGACGTCCTGGCCAAATTCTGGGACGAGTAGCCCA
AACGTCAGACGAGCGCGCTTGTCCCCGAACAAACGACCCACCAATAAAATTATGGTATCC
TATGCCCGCAGAATCTGGACGGACCTGGTTACTGCTTTTTGCGCCGCCTTTTATCCTCTC
CCACCCCCGCGTCCCTGACAAGAATCACAATGAGACCCAAAGTTTGGTTCAGAGGTTTAT
TATGGGCAAACACGGGTAGAAGCGCGCCGCGACACTCACAGATCGTTGACGACCGCCCCG 3000
GCGTAGGAGGTGCTGCGACACTCGAAAAAATTGGTGTGTTTGTCGGTGGACATGAGGCTC
AGCGGAAAGCTGGCGTCGGGGGGTGGGGCGGAAAACAGTGGCTTCATGTGGATAAGGCCC
AACAGGCGATCCGCGCTGAATCGCACGTAGTTTTCGATGGCCGCCAGCGCCGCCGGGCTC
AGGATATGGCTGTCCGTCGGCGCCTGGGATCGGATAAATCCGATCTCGATCTCGACCGCC
TGGCGGAACAGCCCGTACACGCGGTCGGGCGGGGGCTTGGCGTGCCCGCCGAGGTAGTTG 3300
TTGTAGATGTAACACGAGGCCGTCGTGTGCACGGCCTCGTCCCGGCTGATGAGGTCGTTT
GACTGGCAGGTGACCCGCAGAAGGTTGTTGGTGCGAAGGTAGGCGATGGCGGCAAACGAG
GCGGCAAAAAAGATGCCCTCGATGAGGATCATGAGAATGAACTTTTCCGGAACGGAGGCG
CATTCCCGCACCCGCGCTTCCAACCAGTCCACCTTGGCGCGGATGGCCGGGTGGTTGATG
GTACCGGCCACGTACTCGCGGCGCGCCTGGTCGTTGTTGTGGAAAAGCACCAGCTGGATG 3600
ATGTTGTACACGCGCGAGTGTACGACTTCGATGCATTCCTGCTCCACGTAGTAGTGGAGA
ATGTCCTTCTGCTCAAACAGGCCGGAGAGGCCGCCCAGGTTTTCCGTAACCAGGTCGTCG
GCGGCCGACAGGAAAGCGAAGAGGAAGCGGTAAAAGCTGAGCTCGCCCTCGGAAAGCTTG
GAGACGTCCTCCTCGTCCCCCACGAAAACAAGCTCGGTTTCCAGCCAGCGGTTAAGGATG
CTGAGGGAGCGCAGGTGGTTAATGTCGGGACACTGGGAGGTGTAGAAGTACCTCTCGGGG 3900
TCGGGGCACTTTGGAATCTGGATCGCCAGGTCCGCCGTCGCGCTCTGGTCCGTAAGGGCC
GTCAGAGCGGGGGAGAGGGCTGGGGCCGCGGAATCCATGGCAGCAGGGGAGAGCGTGGGA
CGGCGACGACAGTGGCGGCGGGCCTGGCGCGGAGGGGGTTTGTCGGTCACAGCGCGCAGC
TCATGCAGACAATGTTGTCGTCGCCGCCAAAGACCCCGCTGTTGGTCGCCTTGCGAACCT
TGCAGTAGTACATCCCTGTTTTTAGTCCGCGCTTATATGCGTGGACCAGAAGGCGGACCA 4200
GGGTGGAGGCTGGGAGGGTCCCGTCCGCCTTCTCCGTGACATACAGGGTCATGGATTGGC
TATGGT

FIG. 4C

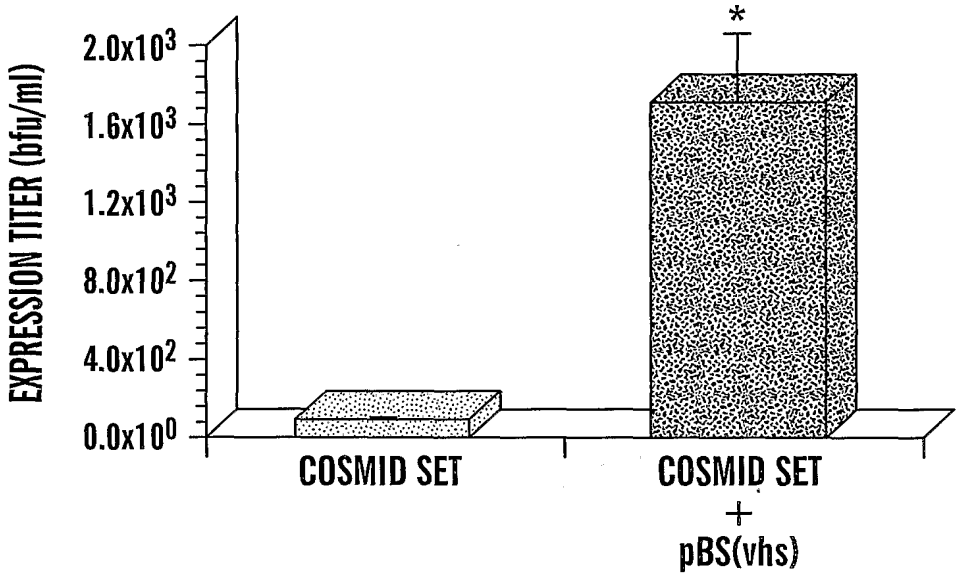

EXPRESSION TITER (bfu/ml)
2.0x10^3
1.6x10^3
1.2x10^3
8.0x10^2
4.0x10^2
0.0x10^0
*
COSMID SET
COSMID SET
+
pBS(vhs)

FIG. 6A

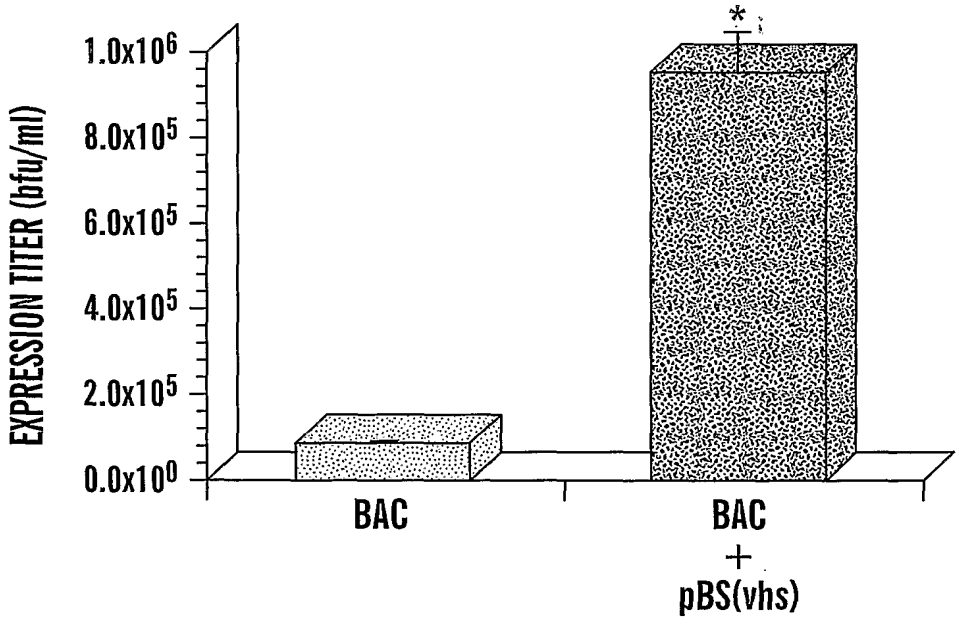

EXPRESSION TITER (bfu/ml)
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0x10^0
*
BAC
BAC
+
pBS(vhs)

FIG. 6B

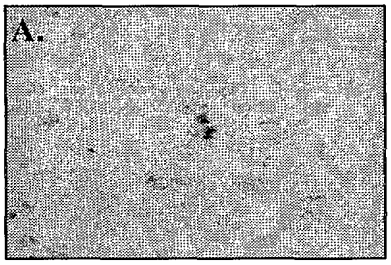


FIG. 7A

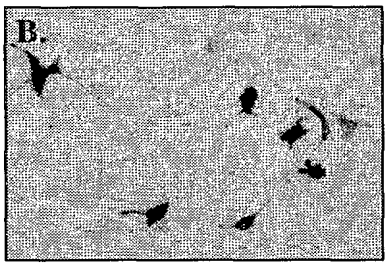


FIG. 7B

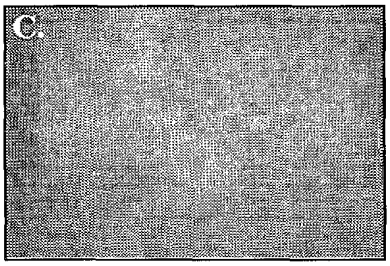


FIG. 7C

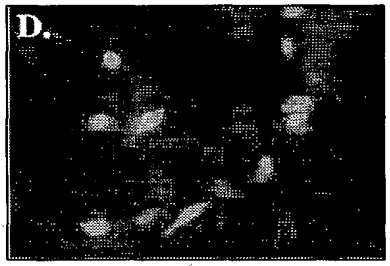


FIG. 7D

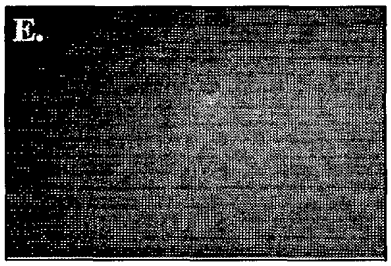


FIG. 7E

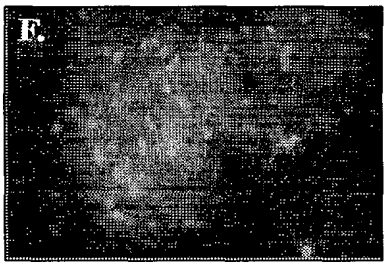


FIG. 7F

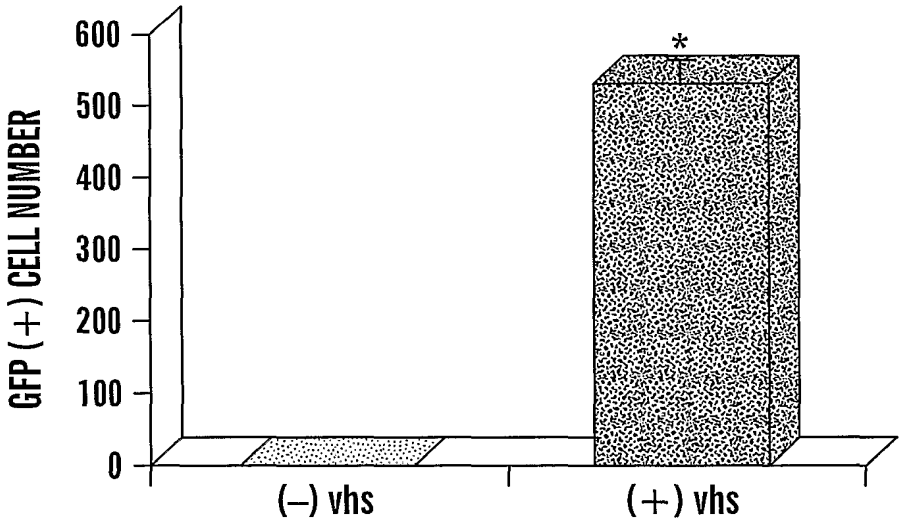


FIG. 7G

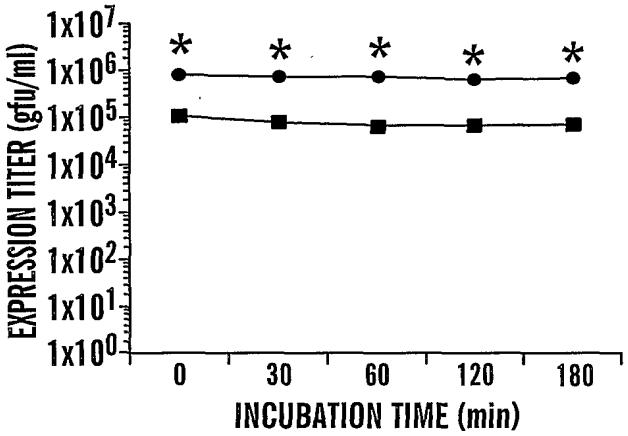


FIG. 8A

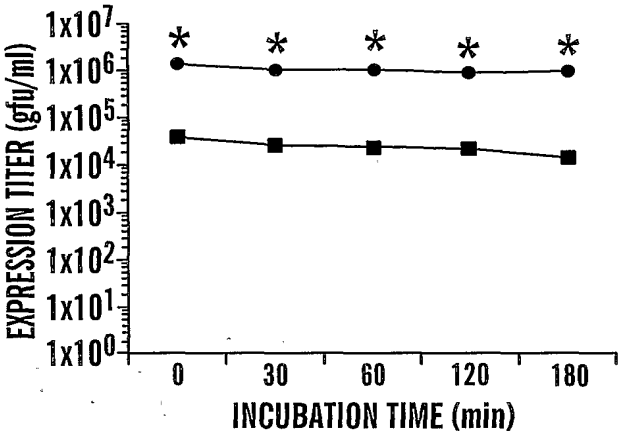


FIG. 8B

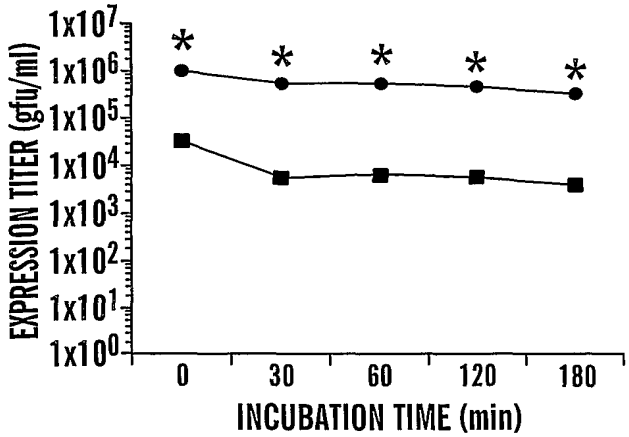


FIG. 8C

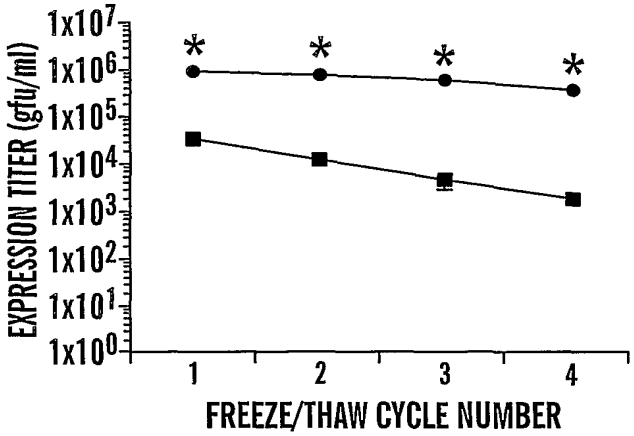


FIG. 8D

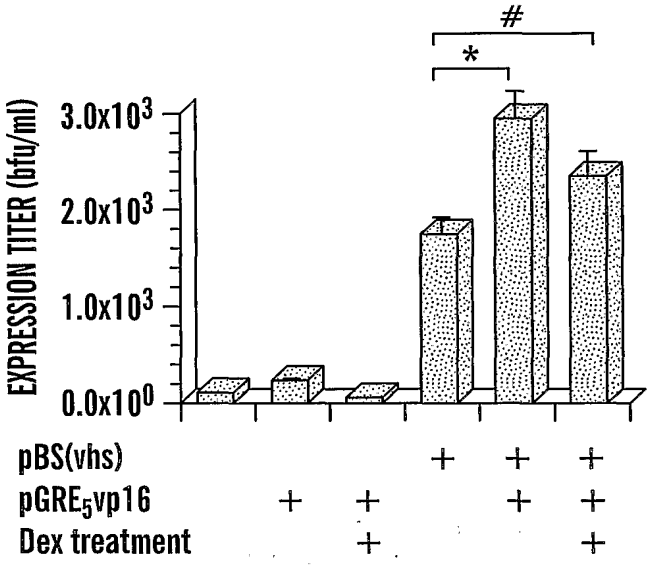


FIG. 9A

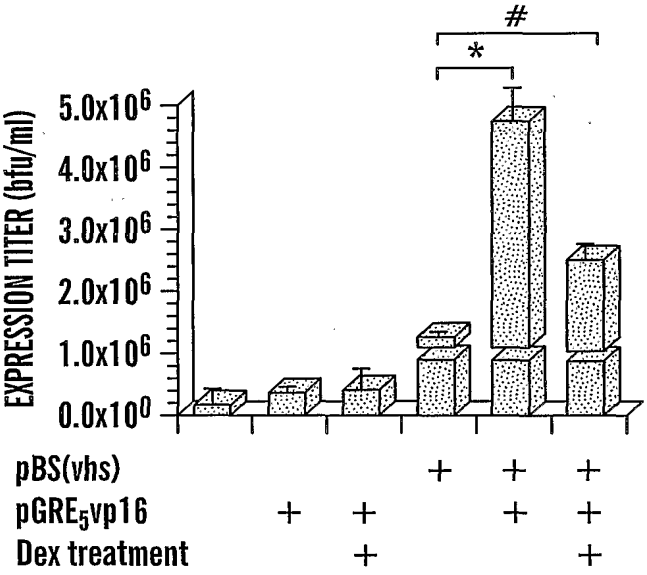


FIG. 9B

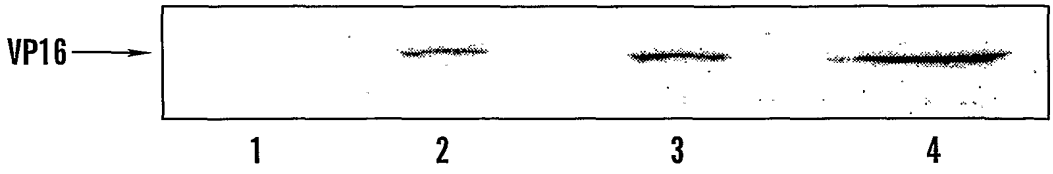
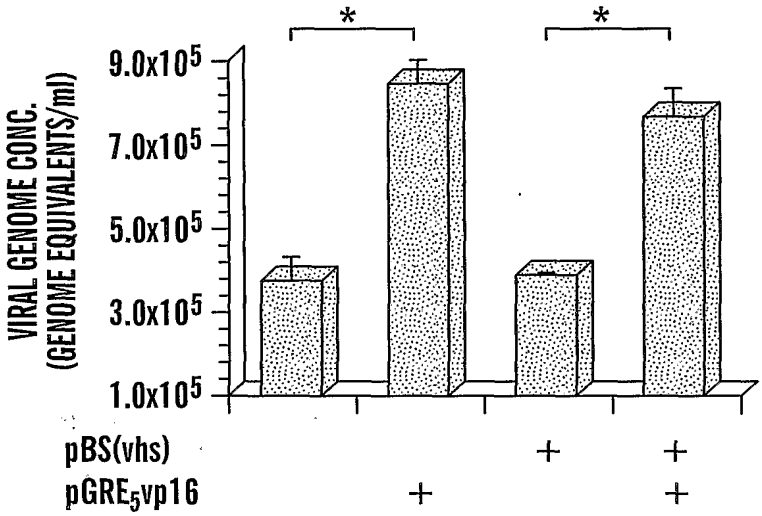


FIG. 9C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*
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(GENOME EQUIVALENTS/ml)
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7.0x10^5
5.0x10^5
3.0x10^5
1.0x10^5
pBS(vhs)
pGRE5vp16
+
+
+
+

FIG. 10

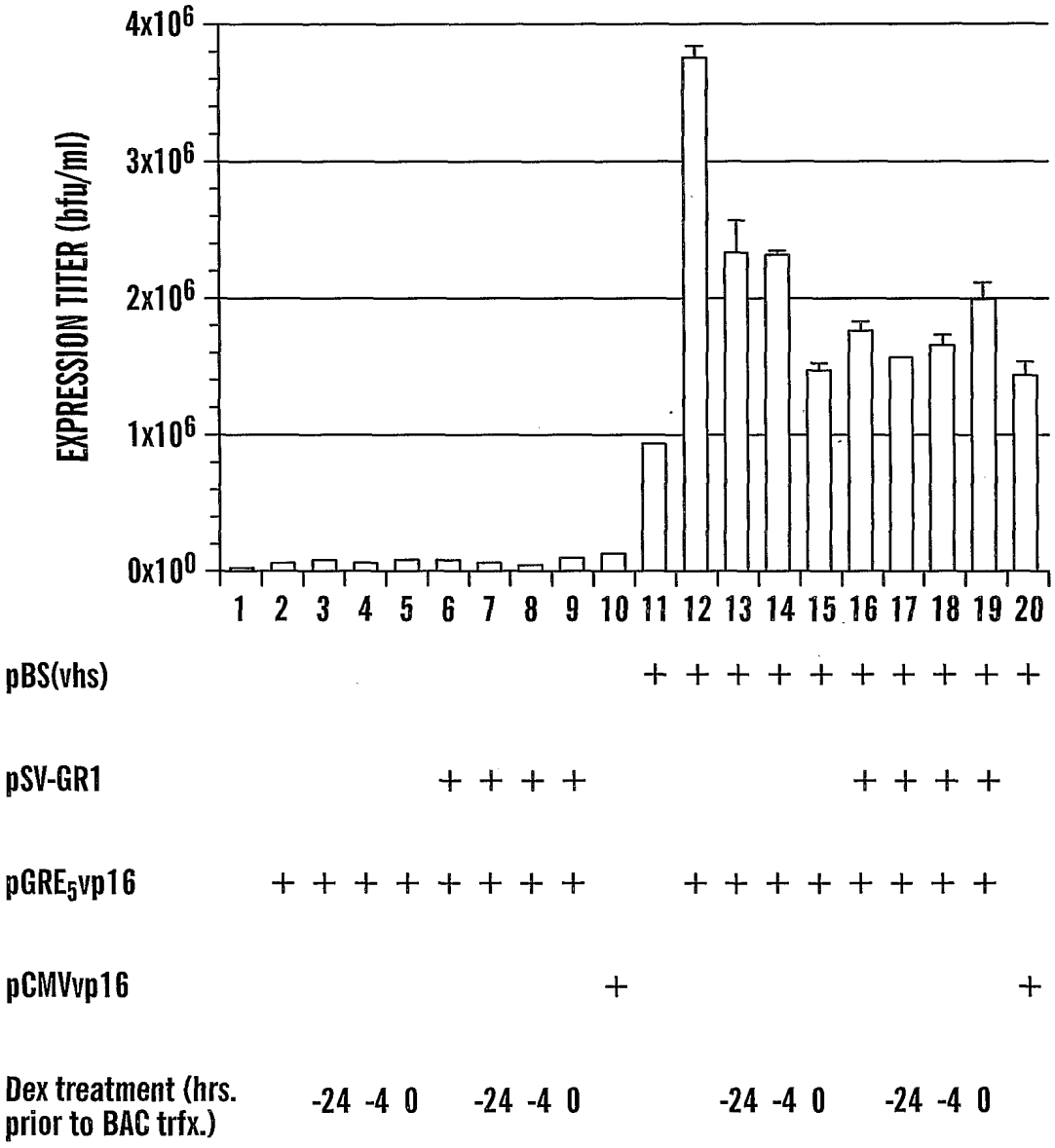
EXPRESSION TITER (bfu/ml)
4x10^6
3x10^6
2x10^6
1x10^6
0x10^0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
pBS(vhs)
pSV-GR1
pGRE5vp16
pCMVvp16
Dex treatment (hrs. prior to BAC trfx.)
-24 -4 0 -24 -4 0 -24 -4 0 -24 -4 0

FIG. 11

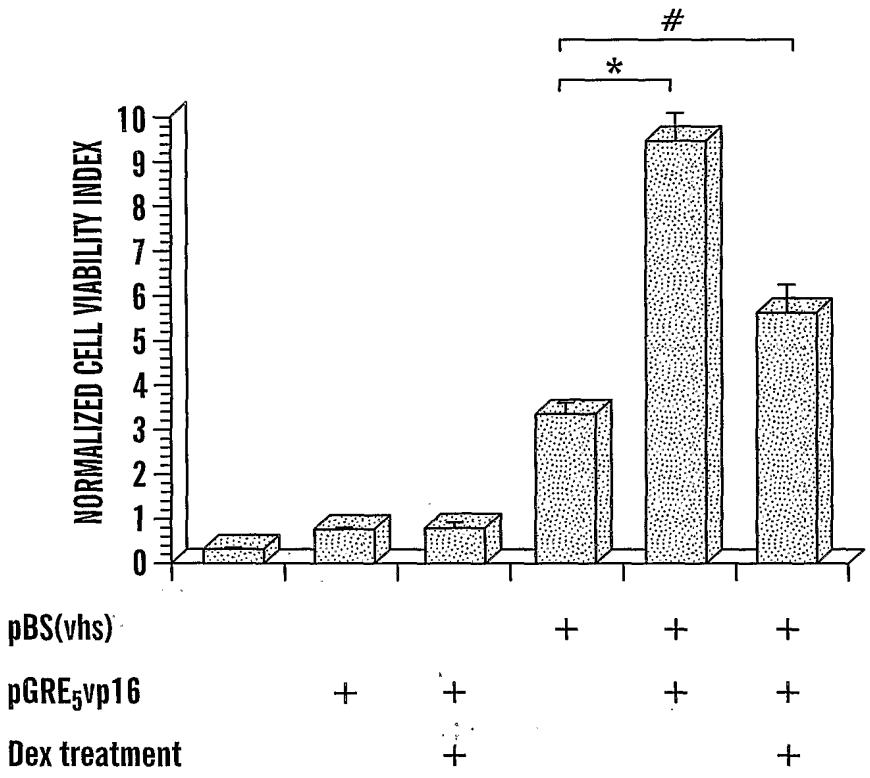
#
*
NORMALIZED CELL VIABILITY INDEX
10
9
8
7
6
5
4
3
2
1
0
pBS(vhs)
pGRE5vp16
Dex treatment
+ + +
+ + + +
+ +

FIG. 12

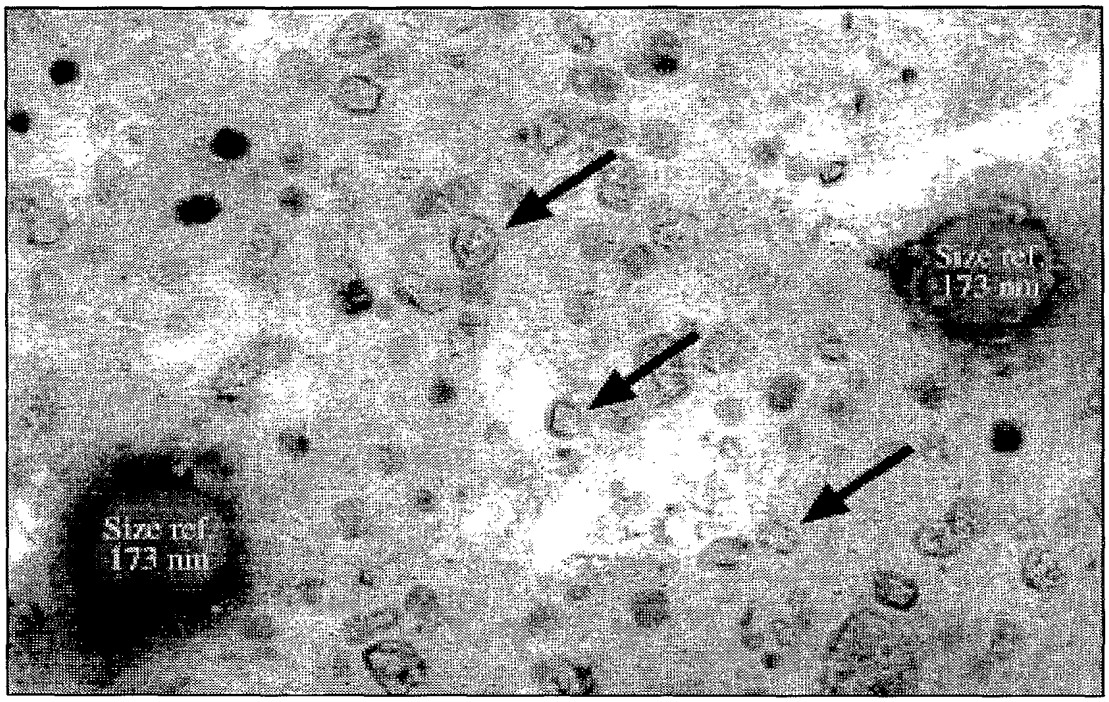

FIG. 13

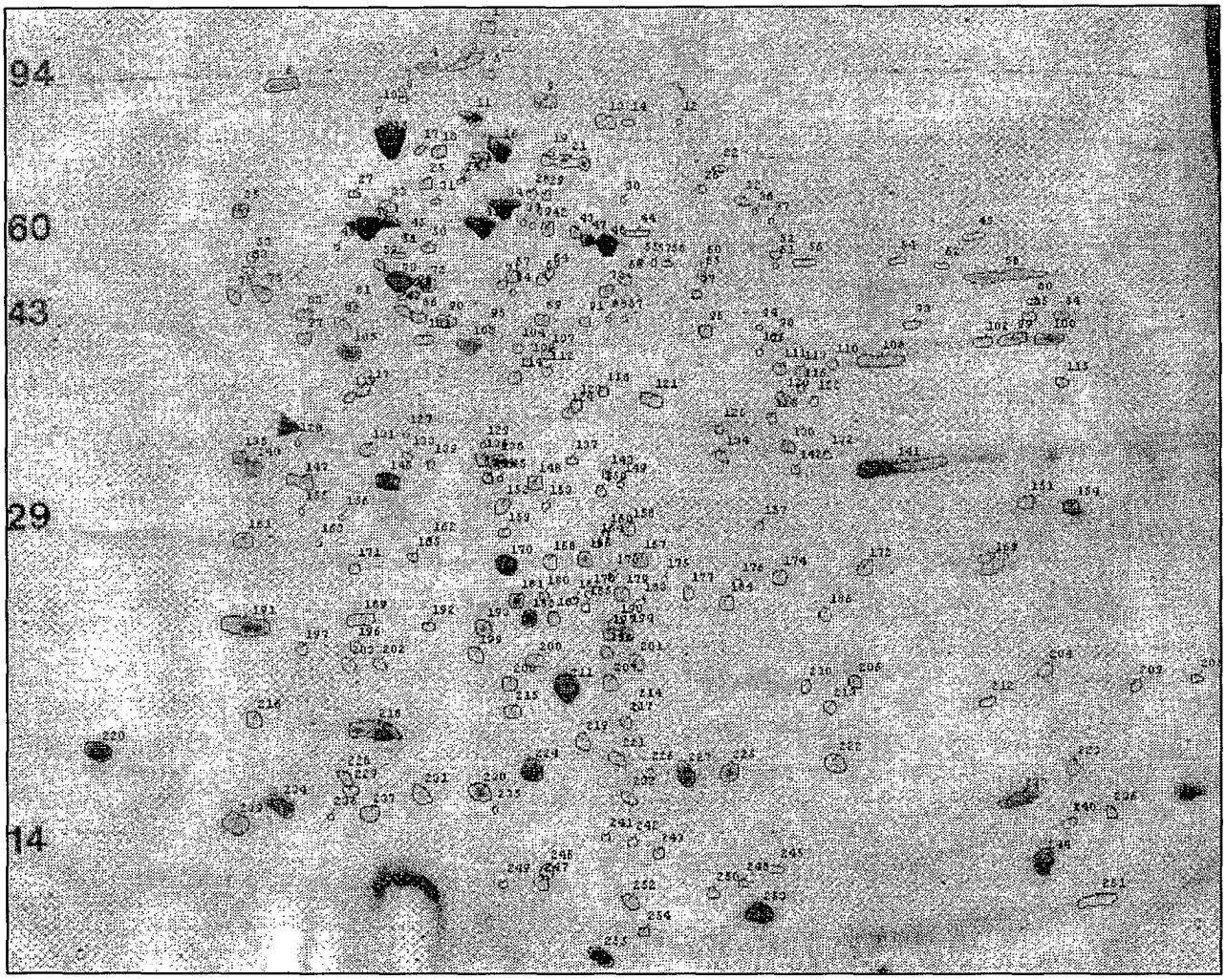

FIG. 14

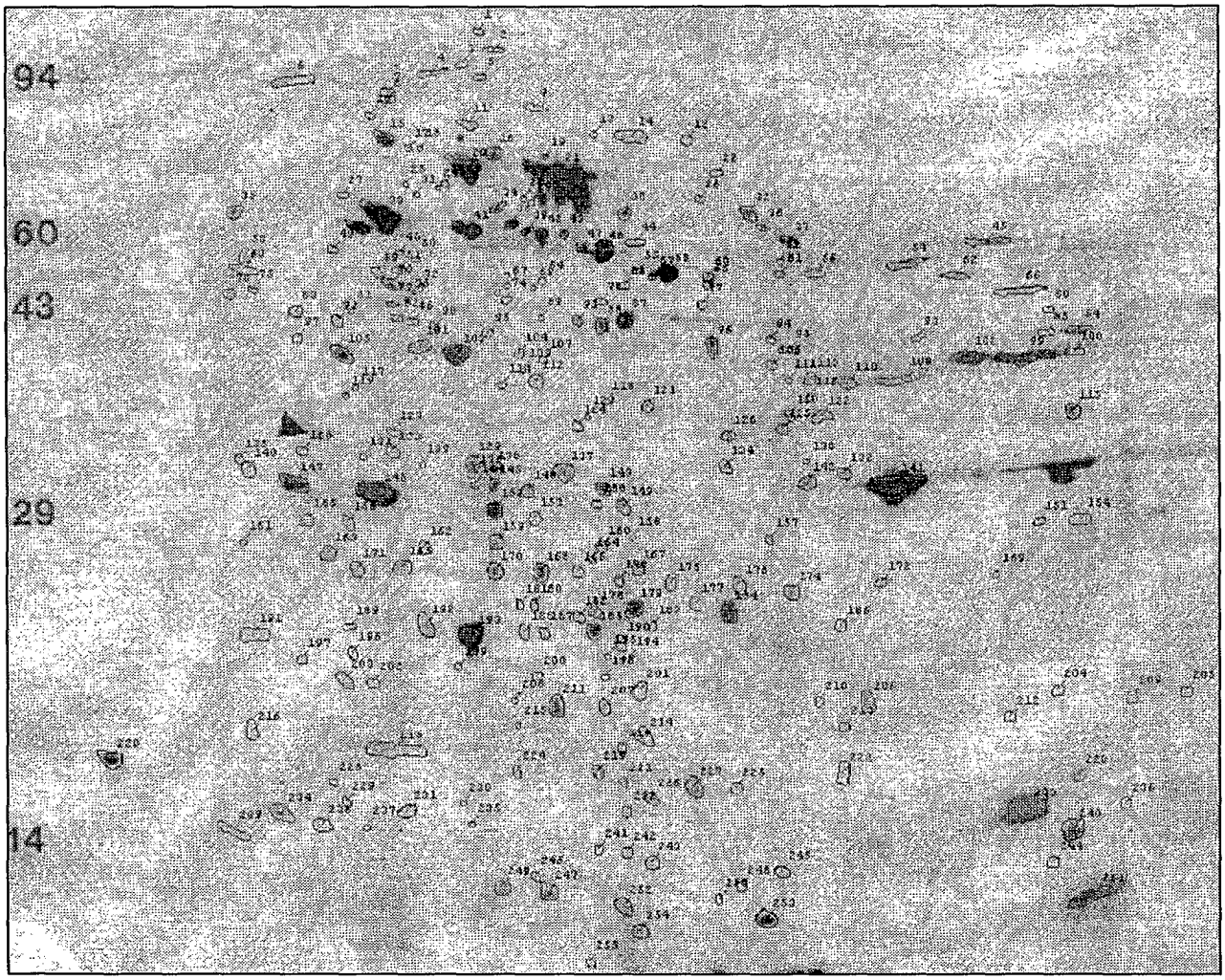

FIG. 15

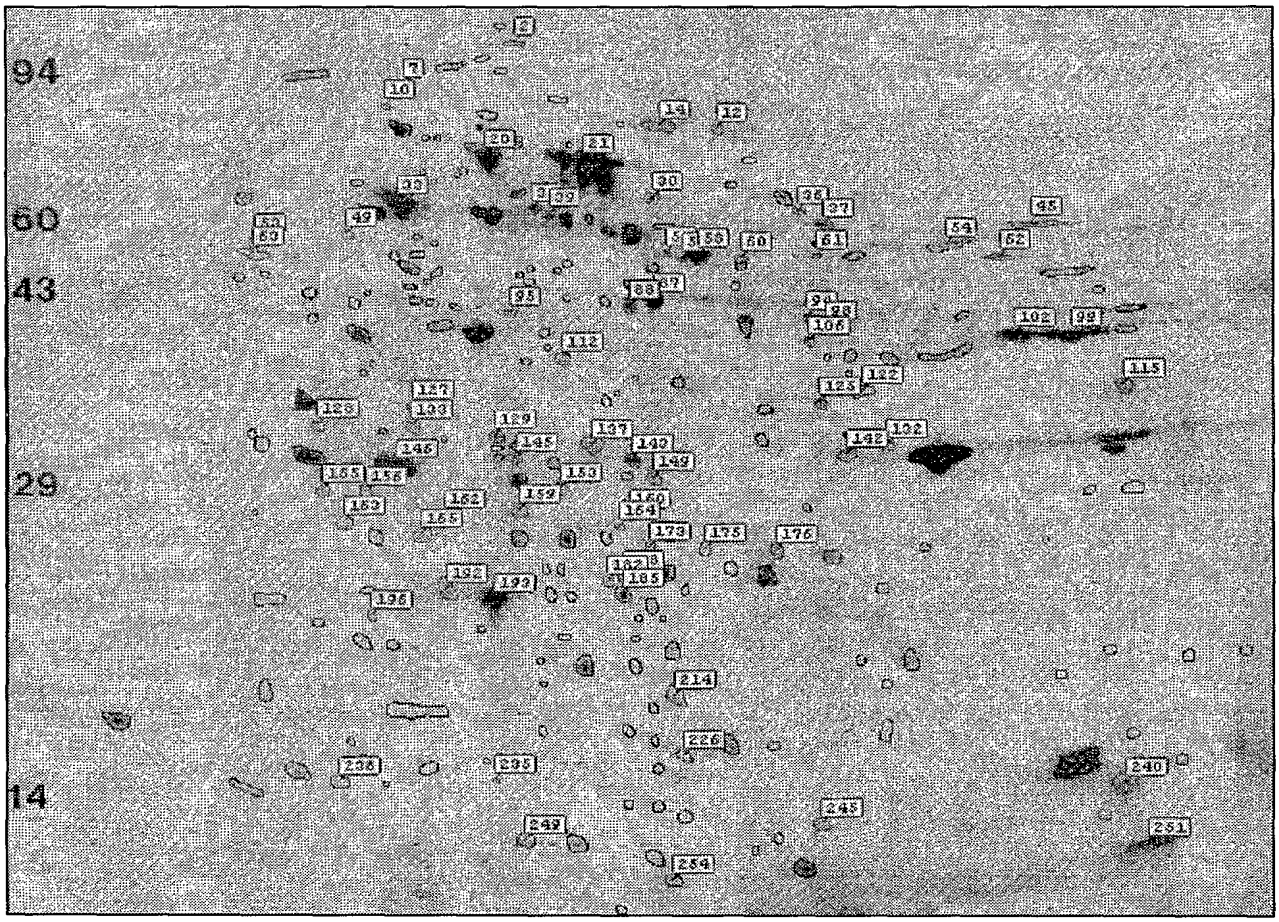

FIG. 16A

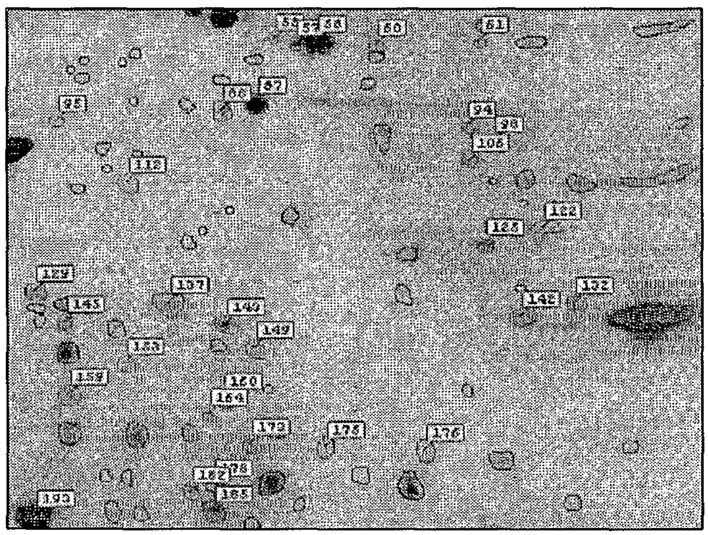
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FIG. 16B

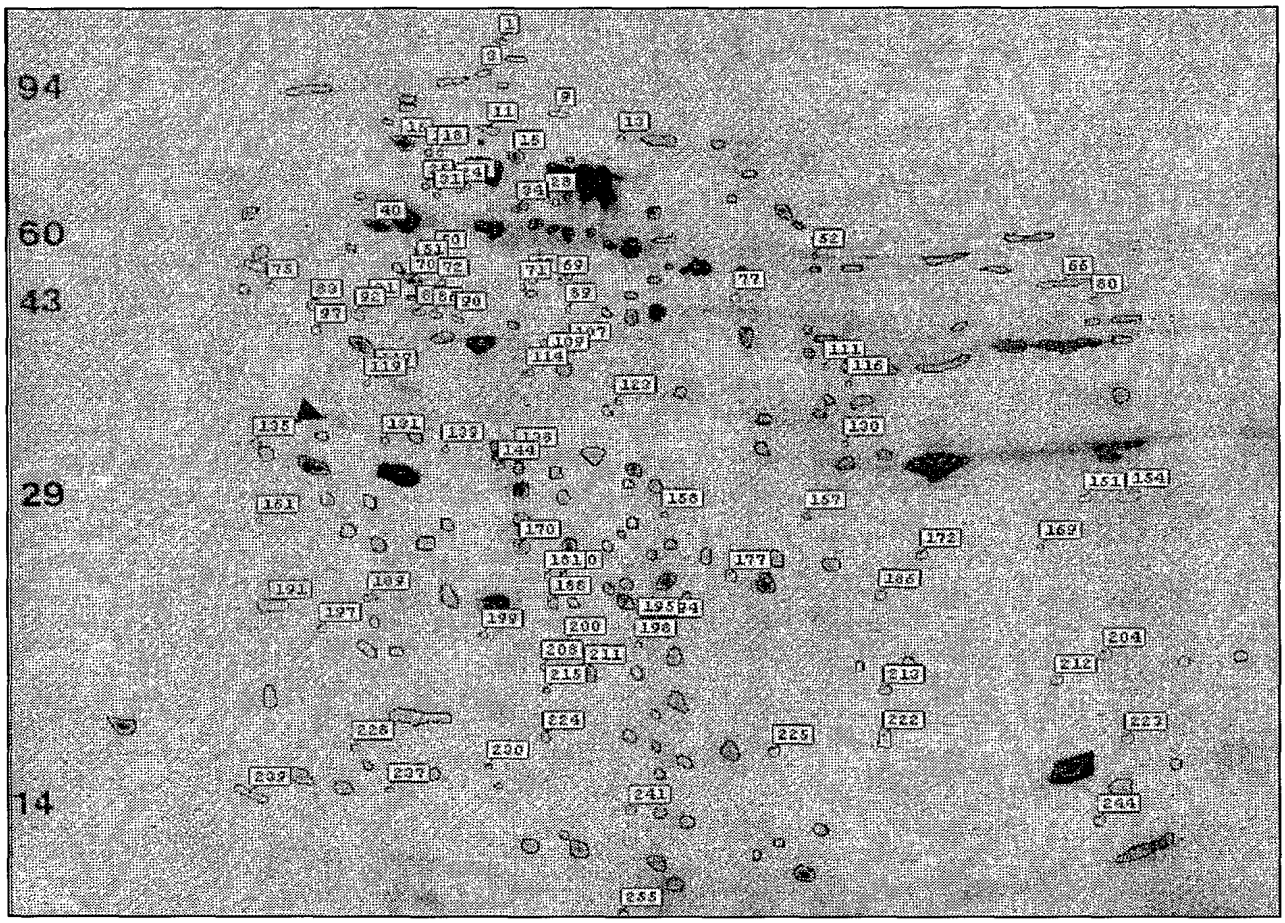

FIG. 17A

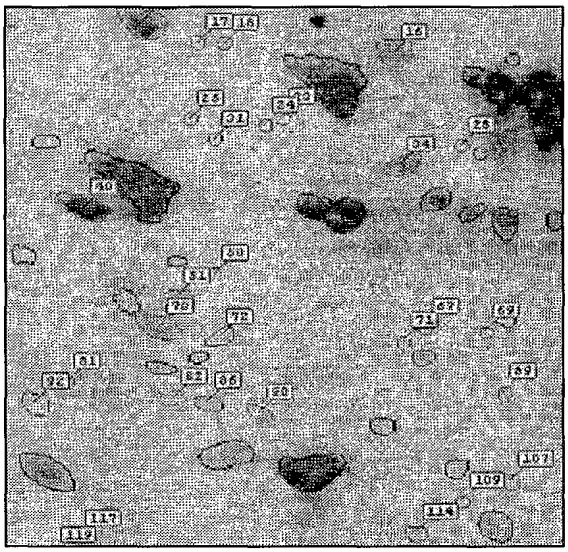

FIG. 17B

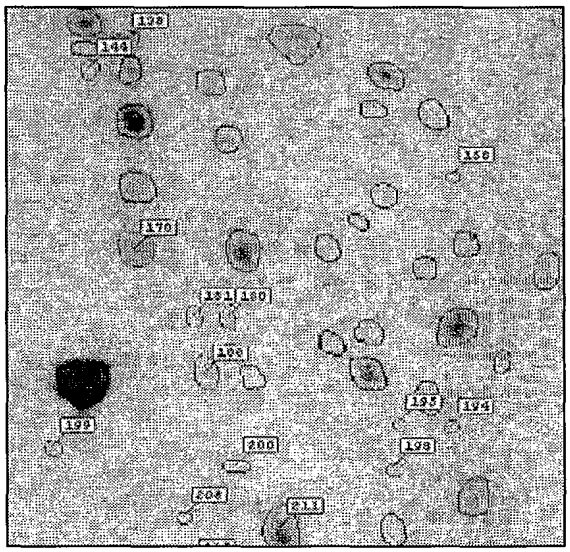

FIG. 17C

SEQUENCE LISTING

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tatggt 4266

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<400> 2

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Lys Arg Arg Gly Leu Gly Ala Pro Ala Gly Tyr Phe Thr Pro Ile Ala
             20                  25                  30

Val Asp Leu Trp Asn Val Met Tyr Thr Leu Val Val Lys Tyr Gln Arg
         35                  40                  45

Arg Tyr Pro Ser Tyr Asp Arg Glu Ala Ile Thr Leu His Cys Leu Cys
     50                  55                  60

Arg Leu Leu Lys Val Phe Thr Gln Lys Ser Leu Phe Pro Ile Phe Val
 65                  70                  75                  80

Thr Asp Arg Gly Val Asn Cys Met Glu Pro Val Val Phe Gly Ala Lys
                 85                  90                  95

Ala Ile Leu Ala Arg Thr Thr Ala Gln Cys Arg Thr Asp Glu Glu Ala
            100                 105                 110

Ser Asp Val Asp Ala Ser Pro Pro Pro Ser Pro Ile Thr Asp Ser Arg
        115                 120                 125

Pro Ser Ser Ala Phe Ser Asn Met Arg Arg Arg Gly Thr Ser Leu Ala
    130                 135                 140

Ser Gly Thr Arg Gly Thr Ala Gly Ser Gly Ala Ala Leu Pro Ser Ala
145                 150                 155                 160

Ala Pro Ser Lys Pro Ala Leu Arg Leu Ala His Leu Phe Cys Ile Arg
                165                 170                 175

Val Leu Arg Ala Leu Gly Tyr Ala Tyr Ile Asn Ser Gly Gln Leu Glu
            180                 185                 190

Ala Asp Asp Ala Cys Ala Asn Leu Tyr His Thr Asn Thr Val Ala Tyr
        195                 200                 205
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Val Tyr Thr Thr Asp Thr Asp Leu Leu Leu Met Gly Cys Asp Ile Val
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Leu Asp Ile Ser Ala Cys Tyr Ile Pro Thr Ile Asn Cys Arg Asp Ile
225                 230                 235                 240

Leu Lys Tyr Phe Lys Met Ser Tyr Pro Gln Phe Leu Ala Leu Phe Val
                245                 250                 255

Arg Cys His Thr Asp Leu His Pro Asn Asn Thr Tyr Ala Ser Val Glu
            260                 265                 270

Asp Val Leu Arg Glu Cys His Trp Thr Pro Pro Ser Arg Ser Gln Thr
        275                 280                 285

Arg Arg Ala Ile Arg Arg Glu His Thr Ser Ser Arg Ser Thr Glu Thr
    290                 295                 300

Arg Pro Pro Leu Pro Pro Ala Ala Gly Gly Thr Glu Thr Arg Val Ser
305                 310                 315                 320

Trp Thr Glu Ile Leu Thr Gln Gln Ile Ala Gly Gly Tyr Glu Asp Asp
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Glu Asp Leu Pro Leu Asp Pro Arg Asp Val Thr Gly Gly His Pro Gly
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Pro Arg Ser Ser Ser Ser Glu Ile Leu Thr Pro Pro Glu Leu Val Gln
        355                 360                 365

Val Pro Asn Ala Gln Leu Leu Glu Glu His Arg Ser Tyr Val Ala Asn
    370                 375                 380

Pro Arg Arg His Val Ile His Asp Ala Pro Glu Ser Leu Asp Trp Leu
385                 390                 395                 400

Pro Asp Pro Met Thr Ile Thr Glu Leu Val Glu His Arg Tyr Ile Lys
                405                 410                 415

Tyr Val Ile Ser Leu Ile Gly Pro Lys Glu Arg Gly Pro Trp Thr Leu
            420                 425                 430

Leu Lys Arg Leu Pro Ile Tyr Gln Asp Ile Arg Asp Glu Asn Leu Ala
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Arg Ser Ile Val Thr Arg His Ile Thr Ala Pro Asp Ile Ala Asp Arg
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Asp Val Leu Ala Lys Phe Trp Asp Glu
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Leu Asp Thr Trp Asn Glu Asp Leu Phe Ser Ala Leu Pro Thr Asn Ala
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Asp Leu Tyr Arg Glu Cys Lys Phe Leu Ser Thr Leu Pro Ser Asp Val
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Val Glu Trp Gly Asp Ala Tyr Val Pro Glu Arg Thr Gln Ile Asp Ile
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Arg Ala His Gly Asp Val Ala Phe Pro Thr Leu Pro Ala Thr Arg Asp
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Gly Leu Gly Leu Tyr Tyr Glu Ala Leu Ser Arg Phe Phe His Ala Glu
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Leu Arg Ala Arg Glu Glu Ser Tyr Arg Thr Val Leu Ala Asn Phe Cys
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Ser Ala Leu Tyr Arg Tyr Leu Arg Ala Ser Val Arg Gln Leu His Arg
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Val Leu Phe Leu His Leu Tyr Leu Phe Leu Thr Arg Glu Ile Leu Trp
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Asp Gly Asp Ser Pro Gly Pro Gly Phe Thr Pro His Asp Ser Ala Pro
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/16682

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A01N 63/00; A61K 48/00; C12N 15/00, 15/09, 15/63, 15/85

US CL :424/93.1, 93.2, 93.6; 435/320.1, 455

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.1, 93.2, 93.6; 435/320.1, 455

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EVERLY, JR. et al. Mutational analysis of the virion host shutoff gene (UL41) of herpes simplex virus (HSV): Characterization of HSV Type 1 (HSV-1)/HSV-2 chimeras. J. Virology. October 1997, Vol. 71, No. 10, pages 7157-7166, entire document.	1-8, 10-70
Y	EVERLY et al. Site-directed mutagenesis of the virion host shutoff gene (UL41) of herpes simplex virus (HSV): Analysis of functional differences between HSV Type 1 (HSV-1) and HSV-2 alleles. J. Virology. November 1999, Vol. 73, No. 11, pages 9117-9129, entire document.	1-8, 10-70

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered *to be of particular relevance*	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be *considered to involve an inventive step when the document is combined* with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 AUGUST 2001	Date of mailing of the international search report 18 OCT 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ANNE-MARIE BAKER, PH.D. Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/16682

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FRAEFEL et al. Helper virus-free transfer of herpes simplex virus type 1 plasmid vectors into neural cells. J. Virology. October 1996, Vol. 70, No. 10, pages 7190-7197, entire document.	1-8, 10-70
Y	US 5,501,979 A (GELLER et al.) 26 March 1996, entire document.	1-8, 10-70
Y	GELLER, A.I. A new method to propagate defective HSV-1 vectors. Nucleic Acids Research. 1988, Vol. 16, No. 12, page 5690, entire document.	1-8, 10-70
Y	GELLER et al. A defective HSV-1 vector expresses Escherichia coli β-galactosidase in cultured peripheral neurons. Science. 23 September 1988, Vol. 241, pages 1667-1669, entire document.	1-8, 10-70
Y	KWONG et al. The herpes simplex virus virion host shutoff function. J. Virology. November 1989, Vol. 63, No. 11, pages 4834-4839, entire document.	1-8, 10-70
Y	MARTUZA et al. Experimental therapy of human glioma by means of a genetically engineered virus mutant. Science. 10 May 1991, Vol. 252, pages 854-856, entire document.	39-70
Y	MELLERICK et al. Physical state of the latent herpes simplex virus genome in a mouse model system: Evidence suggesting an episomal state. Virology. 1987, Vol. 158, pages 265-275, entire document.	1-8, 10-70
Y	PALELLA et al. Herpes simplex virus-mediated human hypoxanthine-guanine phosphoribosyltransferase gene transfer into neuronal cells. Molecular and Cellular Biology. January 1988, Vol. 8, No. 1, pages 457-460, entire document.	1-8, 10-70
Y	STAVROPOULOS et al. An enhanced packaging system for helper-dependent herpes simplex virus vectors. J. Virology. September 1998, Vol. 72, No. 9, pages 7137-7143, entire document.	1-8, 10-70
Y	WIGDAHL et al. Herpes simplex virus latency in isolated human neurons. Proc. Natl. Acad. Sci. USA. October 1984, Vol. 81, pages 6217-6221, entire document.	1-8, 10-70

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/16682

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST
Dialog (file: medicine)
search terms: herpes simplex virus, HSV, amplicon, virion host shutoff, vector, gene transfer, neural, neuron